STUDIES ON PHOTOCHEMICAL REACTIVITY OF DRUG MOLECULES AND RELATED SUBSTANCES

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

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IN

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BY

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DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
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Abstract

The thesis entitled "**studies on photochemical reactivity of drug molecules and related substances,**" describes photochemical studies on drug molecules and related substances and the study of ensuing photochemical processes and mechanisms. Certain drugs are able to produce phototoxicity side effects that are undesired adverse cutaneous reactions that appear as a consequence of the combined action of drugs and sunlight in the treated patients. The variety in molecular structure of phototoxic drugs is immense and almost all classes of drug compounds contain members with adverse photobiological effects. Also, photochemical degradation, by various procedures, including photooxidation, is likely to be an important loss and toxicity mechanism for many drugs and pharmaceuticals in biosystem, and this is of great significance to environmental photochemistry and photobiology. Natural products have served as tools and leads for the development of new drugs and several natural compounds from plants and animal kingdom are now useful drugs. Moreover, plenty of plant materials for their biologically active principal have proved to be of potential medicinal value.

Singlet oxygen, a potential product of photochemical reactions of many compounds is a damaging agent to all living organisms, and also plays a very significant role in plant metabolism. Photosensitizing reactions among secondary plant products is a wide spread phenomenon involving ultra-violet as well as visible light. Secondary plant substrates of diverse biogenetic origin are capable of the photogenerating singlet oxygen, and they also act as quenchers of singlet oxygen, suggesting the wide spread

use of these as potent toxic, protective and defensive agents. Thus, photochemical studies on drugs and promising drug molecules is an area of vital importance in current medicinal chemistry, for establishing a relation to its phototoxicity. A satisfactory understanding of this phenomenon requires a detailed knowledge of the photochemistry of such molecules. In principle, photochemical reactivity of drugs can be anticipated on the basis of good knowledge of the possible photochemical mechanisms. To achieve this goal different types of studies have to be undertaken:

(a) Photophysical studies– light absorption and emission (fluorescence, phosphorescence) to determine the nature of involved excited states, as well as their energies. Laser flash photolysis for detection of triplet states of other short-lived transition species that could interact with biomolecules. Singlet oxygen detection (steady state or time resolved near infrared emission).

(b) Photosensitized reactions of biomolecules– photodynamic lipid peroxidation, photomodification of proteins (protein photocrosslinking, drug protein photobinding) drug photosensitizer DNA damage (strand breaks, oxidative damage to bases, pyrimidine dimers).

(c) Photochemical studies– photostability, photodegradation (isolation and identification of drug-derived photoproducts by chromatography and spectroscopy, product-based elucidation of the photochemical mechanisms.

(d) Photooxidation of drug molecules with singlet oxygen.

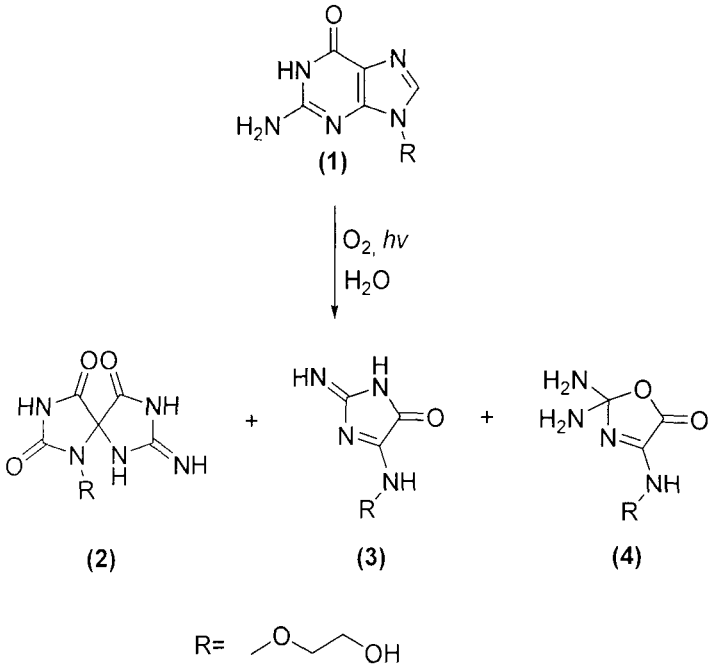
In the present work we have undertaken basically two of the above aspects 'c' and 'd' of the drug photochemistry, using some representative examples from established drugs and promising drug (biologically active molecules) class.

Chapter 1 of the thesis includes some basic concepts of photochemistry; some active areas of photobiological research with an outline of their importance; properties, generation and reaction of singlet oxygen; some facts pertinent to human photobiology and a brief survey of the photochemistry and photobiology of drugs and some biologically active natural products.

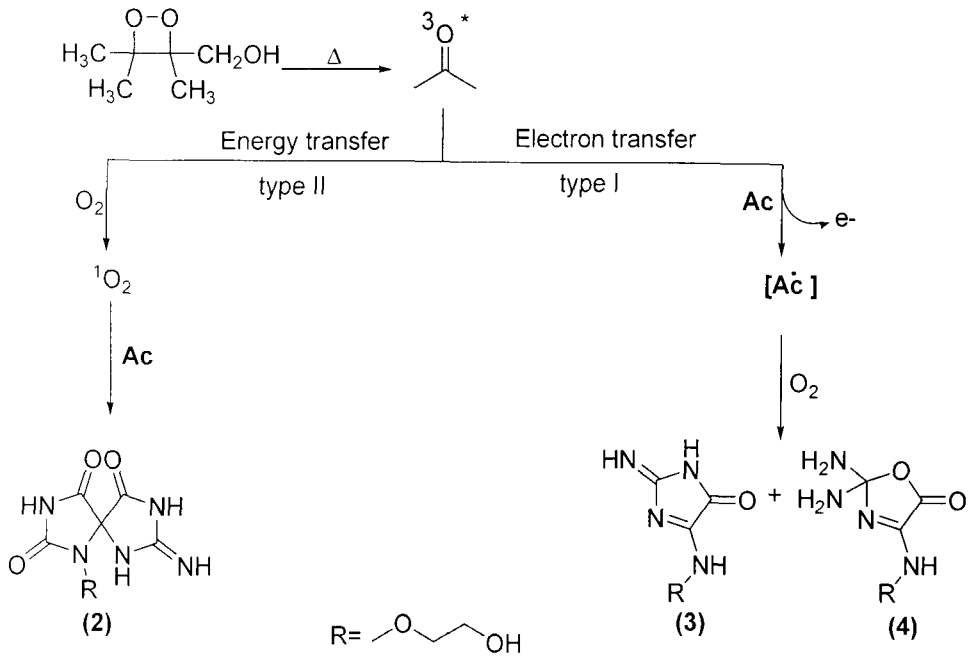
Second chapter of thesis describes photochemical studies on acyclovir **(1)** and phenazopyridine hydrochloride. Photodegradation of aqueous solution of acyclovir in phosphate buffer (pH 7) under aerobic condition was studied with light of wavelength >270 nm. Three major products were isolated and identified on the basis of IR, NMR and mass spectral studies. The products were: (2-hydroxyethoxy) methyl spiroiminodihydantoin **(2)**, (2-hydroxyethoxy) methyl (amino)-2-imino-1,2-dihydroimidazole-5-one **(3)**, and 2,2-diamino-4-[(2-hydroxyethoxy) methyl) amino)-5-[2H]-oxazolone **(4)**. (Scheme 1) Further the effects of: D_2O as reaction medium, added sodium azide and the absence of oxygen on the photodegradation of acyclovir was checked. These observations indicated the involvement of singlet oxygen. The formation of products is explained by the photooxidation of acyclovir.

In an extensive study the antiviral drug acyclovir (**1**) was treated with triplet excited ketone, generated by thermal decomposition of 3-(hydroxymethyl)-3,4,4-

trimethyl-1,2-dioxetane (**HTMD**), in the dark. Three major photo-oxidation products were isolated and characterized by spectroscopic studies. The products were (2-hydroxyethoxy) methyl spiroiminodihydantoin **(2)**, (2-hydroxyethoxy) methyl (amino)-2-imino-1,2-dihydroimidazole-5-one **(3)**, and 2,2-diamino-4-[(2-hydroxyethoxy) methyl) amino)-5-[2H]-oxazolone **(4)**. The HTMD-induced reaction was found to occur by two photooxidation mechanisms, at almost equal pace: type I leading to **3** and **4** and type II giving **2**. The findings are based upon determination of the exact yields of products in a comparative study with the established photosensitizers, riboflavin (type I) and rose Bengal (type II) (Scheme 2).

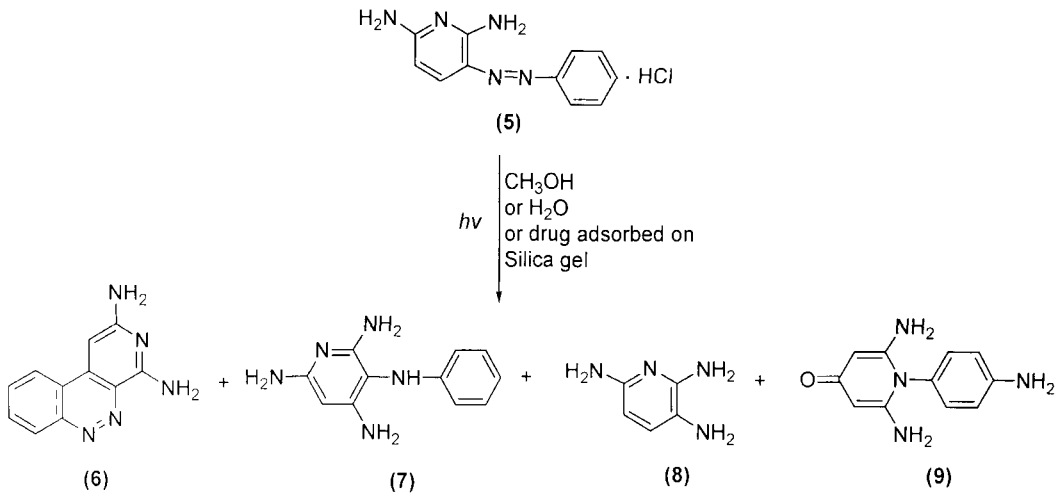


Scheme 1



Scheme 2

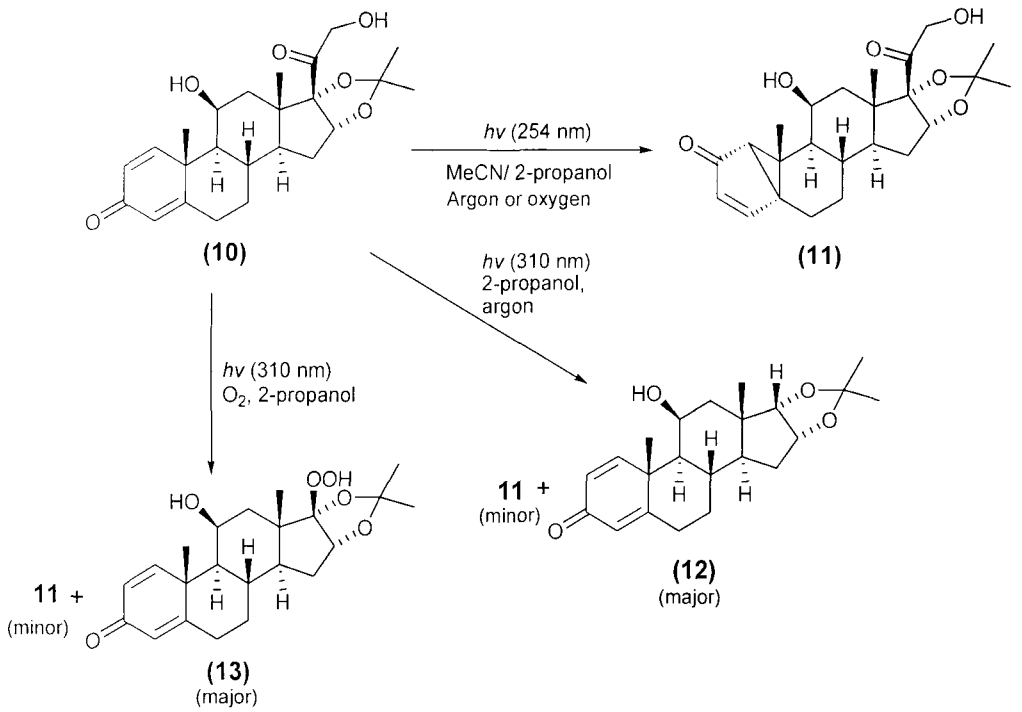
Photochemistry of phenazopyridine hydrochloride **(5)** was studied in different reaction media including the drug adsorbed on silica gel. This resulted in photochemical cyclodehydrogenation, reductive photodegradation and rearrangement of the drug molecule. Four major products were isolated and identified on the basis of IR, NMR and mass spectral studies. The products were: pyrido[3,4-c]cinnoline-2,4-diamine **(6)**, N^3-phenylpyridine-2,3,4,6-tetraamine **(7)**, pyridine-2,3,6-triamine **(8)**, 2,6-diamino-1-(4-aminophenyl)pyridin-4(1H)-one **(9)** (Scheme 3).



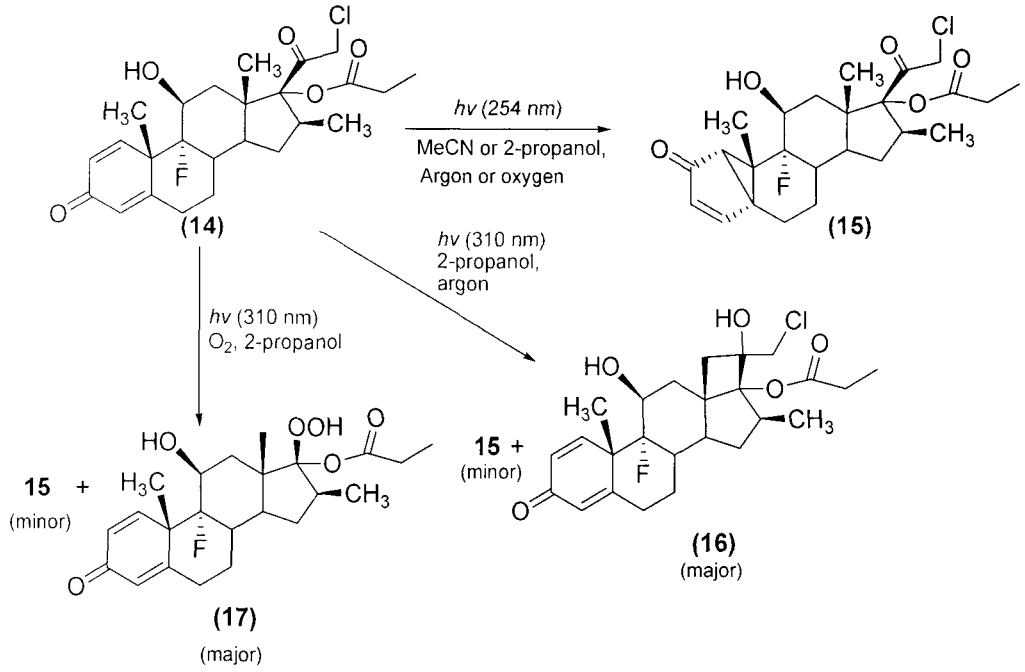
Scheme 3

Chapter 3 of the thesis records the photochemistry of two anti-inflammatory drugs desonide (**10**) and clobetasol propionate **(14)** was studied in aerobic as well as in anaerobic conditions with different irradiation wavelengths (254 nm and 310 nm) in acetonitrile and 2-propanol. The photoproducts were characterized on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral and elemental analysis studies. The products from the photolysis of desonide were: 11β, 21-dihydroxy-16α, 17α-(1-methylethylidenedioxy)-1,5-cyclopregn-3-ene-2,20-dione **11**, 11β-hydroxy-16α, 17α-(1-methylethylidenedioxy) androsta-1,4-diene-3-one **12**, 17β-hydroperoxy-11β-hydroxy-16α, 17α- (1-methylethylidenedioxy) androsta-1,4-diene-3-one **13** (Scheme 4). From the photolysis of clobetasol **(14)** products were: 21-chloro-9-fluoro-11-hydroxy-16-methyl-17(1-oxopropoxy)-1,5-cyclopregn-3-ene-2,20-dione **15** (254 nm),

21-chloro-9-fluoro-11-hydroxy-16-methyl-17(1-oxopropoxy)-18,20-cyclopregn-1,4-diene-3-one **16**, 9-fluoro-17-hydroperoxy-16-methyl-17(1-oxopropoxy) androsta-1,4-diene-3-one **17** (Scheme 5).

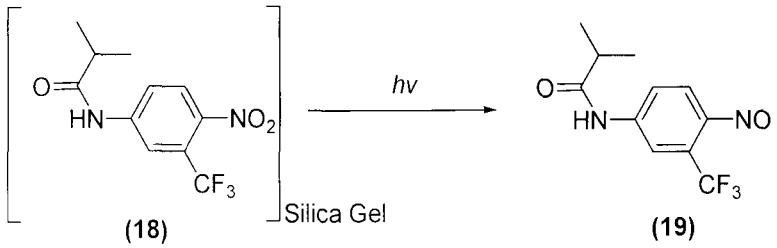


Scheme 4



Scheme 5

The chapter 4 records our investigation on the photoreactivity of anti-cancer drug flutamide (**18, FM**) adsorbed on silica on the silica gel TLC plates. The results were consistent with the formation of nitroso derivative **19** as the sole stable photoproduct, formation of which was realized by the structural changes of flutamide occurring upon its compartmentalization in the silica gel intermolecular cavities (Scheme 6).



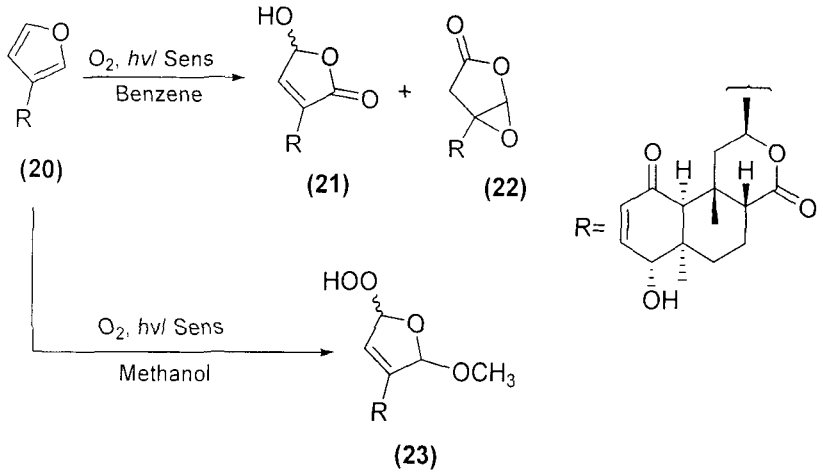
Scheme 6

The photostability of three flutamide oral dosage formulations (tablets available in India) were studied using indirect sunlight (daylight) and continuous artificial light. The extent of photodecomposition of **FM** was determined using a specific reversed phase high performance liquid chromatography (HPLC) method. The effectiveness of artificial and natural sunlight on **FM** photodegradation was also determined using both pure **FM** powder as well as a methanolic **FM** solution for comparison to tablet form. All the tested **FM** formulations were likely to be photostable up to at least 12 weeks of continuous artificial and natural day light exposure, compared with pure **FM** powder and methanolic solution. Photodegradation of **FM** powder and methanolic solution exposed to indirect sunlight was faster than the artificial light.

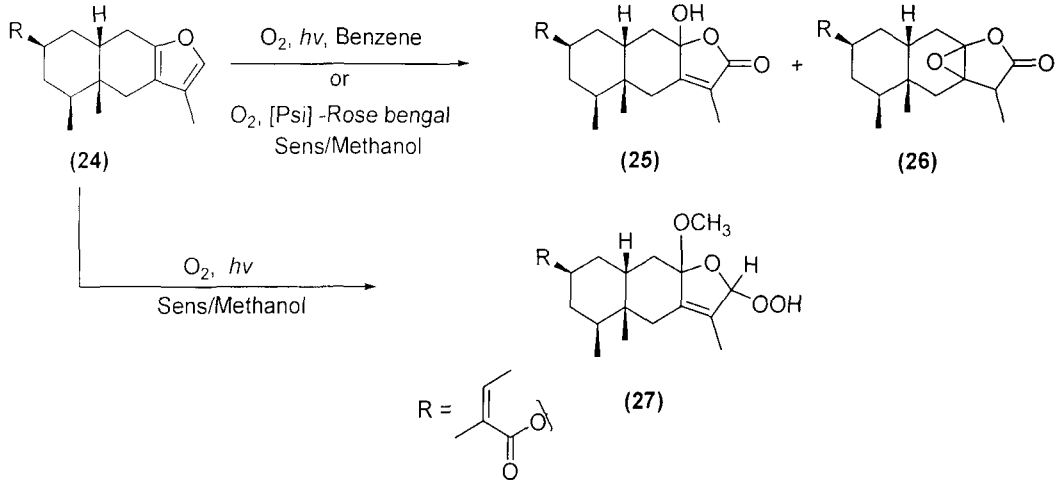
Chapter 5 of the thesis describes photooxygenation reaction of two plant-derived terpenoids tinosponone **(20)** and furanoeremophilane **(24)**. The reaction of tinosponone with singlet oxygen was studied by using different combinations of photosensitizers (i.e. rose bengal, methylene blue, riboflavin and benzophenone), solvents (i.e. benzene, chloroform, acetone, acetonitrile and methanol) and singlet oxygen scavengers (i.e. DABCO and sodium azide). Two major products: (3S,4aS,4bS,8R,8aR,10aR)-8-hydroxy-3-(5′-hydroxy-2′-oxo-2′,5′-dihydrofuran-3′-yl)-4a,8a-dimethyl-3,4,8,8a,9,10-hexahydro-10aH-benzo[f]isochromene-1,5 (4aH,4bH)-dione **(21)** and (3S,4aS,4bS,8R,8aR,10aR)-8-hydroxy-4a,8a-dimethyl-3-((1′R)-3′-oxo-4′,6′-dioxa-bicyclo[3.1.0]hexan-1′-yl)-3,4,8,8a,9,10-hexahydro-10aH-benzo[f] isochromene-1,5(4aH,4bH)-dione **(22)** were isolated in all the solvents except methanol. In methanol a single product (3S,4aS,4bS,8R,8aR,10aR)-8-hydroxy-3-

(5′-hydroperoxy-2′-methoxy-2′,5′-dihydrofuran-3′-yl)-4a,8a-dimethyl-3,4,8,8a,9,10-hexahydro-10aH-benzo[f] isochromene-1,5(4aH,4bH)-dione **(23)** was obtained (Scheme 7). All products were characterized on the basis of IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral and elemental analysis studies. The formation of products was explained by photooxidation of tinosponone. Effect of different solvents with the variation of added singlet oxygen sensitizers and singlet oxygen scavengers was observed on the yields of photooxidation products and was correlated to the rate of singlet oxygen formation.

The photochemical oxygenation reaction of 2β-angeloyloxy-10β-H-furanoeremophilane **(24)**, a sesquiterpene, was studied in benzene and methanol. Three photoproducts were isolated and characterized by IR ^{1}H-NMR, ^{13}C-NMR and mass spectral studies. Sesquiterpene itself was found to be singlet oxygen ($^{1}O_2$) sensitizer. Addition of rose bengal increased the rate of photooxidation whereas as DABCO was found to decrease the rate of photolysis proving the involvement of $^{1}O_2$ in these photoreactions. 2β-angeloyloxy-8-hydroxy –10β-H-eremophilanolide **(25)** and 2β-angeloyloxy–7,8-epoxy-10β-H-eremophilanolide **(26)** were obtained as products in benzene. Photolysis in methanol gave a single product 2β-angeloyloxy-10β-H-8-methoxy-12-hydroxy-7,11–dihydro-eremophilanolide **(27)**. Reaction was also carried out by adsorbing compound **(24)** on silica gel bound rose bengal which yielded the products **25** and **26** with an increase in the rate of reaction (Scheme 8).



Scheme 7



Scheme 8

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Dedicated to

my family

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Certificate

Certified that the work embodied in this thesis entitled "**Studies on Photochemical Reactivity of Drug Molecules and Related Substances**" is the result of original researches carried out under my supervision by **Mr. Adil Husain** and is suitable for submission for the award of Ph.D. degree of Aligarh Muslim University, Aligarh, India.

(Dr. Jawaid Iqbal)

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Adil Husain

List of Publications

- Photooxidation of acyclovir in aqueous solution, Jawaid Iqbal, Adil Husain, Anamika Gupta, *Die* ***Pharmazie*** **2005,** 60, 574-576, ***Govi-Verlag, Germany***.

- Sensitized photooxygenation of tinosponone, a clerodane diterpene from *Tinospora cordifolia*, Jawaid Iqbal, Adil Husain, Anamika Gupta, ***Acta Chim. Slov.*** **2005**, 52 (in Press), ***Slovenian Chemical Society.***

- Photomediated transformation of eremophilanes-I: Photooxidation of 2β-angeloyloxy-10β-H-furanoeremophilane, Jawaid Iqbal, Anamika Gupta, Adil Husain, (Accepted) ***ARKIVOC, Arakat-USA.***.

- Photooxidation of acyclovir by thermally generated triplet excited ketone from 1,2-dioxetane and comparison with type I and type II photosensitizers, Jawaid Iqbal, Adil Husain, Anamika Gupta, (Revised manuscript submitted) ***Chemical and Pharmaceutical Bulletin, Japan***.

- Photochemistry of desonide, a non-fluorinated steroidal anti-inflammatory drug, Jawaid Iqbal, Adil Husain, Anamika Gupta, (Communicated) ***Photochemical Photobiological Sciences, Royal Chemical Society.***

- Photolysis of anticancer drug flutamide adsorbed on silica Jawaid Iqbal, Adil Husain, Anamika Gupta, (Communicated) ***Journal of Pharmacy and Pharmaceutical Sciences, Canada***.

- Photochemistry of clobetasol propionate, a steroidal anti- inflammatory drug, Jawaid Iqbal, Anamika Gupta, Adil Husain, (Communicated) ***Bulletin Chemical Society of Japan.***

- Photochemistry of Phenazopyridine Hydrochloride, Jawaid Iqbal, Anamika Gupta, Adil Husain, (Communicated) *Die* ***Pharmazie Govi-Verlag,, Germany.***

- Photostability determination of commercially available flutamide oral dosage formulations. (Under preparation)

CONFERENCE ATTENDED:

- "Sensitized Photooxygenation of Tinosponone, a Clerodane Diterpene fromTinospora cordifolia" Adil Husain and Jawaid Iqbal, Presented at national conference on emerging trends in chemical sciences in the new millennium, Organized by P. G. Department of Chemistry and research center, Shri Shivaji Science college, Amravati, India, Feb. 5-7, 2004.

CONTENTS

Chapter 1

General Introduction

Light is a ubiquitous element of our environment with a tremendous impact on life. Without light, life, as we know it would not exist. Plants harvest solar energy by photosynthesis, and provide energy to other organisms through the food chain. Humans and animals depend on light through vision and other photoresponses. Biological effects of artificial light are the basis of a variety of medical treatments and diagnostic techniques. Light can also have deleterious effects. For example, ozone in the atmosphere protects living things from damage by ultraviolet radiation. Increases in the amount of ultraviolet light reaching the earth's surface as a result of changes in the ozone layer may result in increased rates of skin cancer and skin aging as well as undesirable effects on agriculture, oceanic plankton and the aquatic food chain. Recognition of the importance of light in biology has led to the development of the science of photobiology: the study of the myriad effects of light on life.

There is growing awareness of the influence of light (both beneficial and harmful) on living organisms.[1] Some active areas of photobiological research and an outline of their importance to society is highlighted in the following section.

Photosynthesis

Photosynthesis is one of the most important biological processes on earth. By consuming carbon dioxide and liberating oxygen, it has transformed the world into the hospitable environment we know today. Directly or indirectly, photosynthesis fills all of our food requirements and many of our needs for fiber and building materials. The energy stored in petroleum, natural gas and

coal all came from the sun via photosynthesis, as does the energy in firewood, which is a major fuel in many parts of the world. This being the case, scientific research into photosynthesis is extremely important. If we can understand and control the intricacies of the photosynthetic process, we can learn how to increase crop yields of food, fiber, wood, and fuel, and how to use our lands more benignly. The energy-harvesting secrets of plants can be adapted to synthetic systems, which will provide new, efficient ways to collect and use solar energy. Because photosynthesis helps control the makeup of our atmosphere, understanding photosynthesis is crucial to understanding how carbon dioxide and other 'greenhouse gases' affect the global climate.

Photomedicine

To avoid the sun, would be to exist without one of the great pleasures of life. But as with most enjoyable things, indiscriminate exposure and lack of understanding of the possible unpleasant consequences can result in unhappiness and even serious aftereffects. There are over 25 diseases that are caused or aggravated by sunlight. The field of photomedicine includes the study of such diseases and their treatments.

Harmful effects of light - Sunlight is implicated in several skin diseases, including premature aging of the skin and skin cancer. Skin sensitivity to sunlight is controlled by the genetic ability of an individual to produce melanin, the pigment that helps protect the skin from light-induced injury. Genetic variations in the capacity to form melanin, proximity to the equator, and personal habits of sun exposure determine the susceptibility of an

individual to skin aging and cancer of the skin. Deficiencies in cellular capacity to repair sun-induced damage of DNA, as in the inherited disorder called xeroderma pigmentosum, are responsible for the early onset of sun sensitivity and freckling, which can lead to sunlight-induced skin cancer. Certain drugs or chemicals also augment skin reactivity to solar radiation, and lead to transient phototoxic effects or chronic photoallergic reactions. In the synthesis of hemoglobin, the substance that gives the red color to human blood and carries oxygen, genetic deficiencies of certain enzymes lead to metabolic overproduction of hemoglobin precursors called porphyrins. These porphyrins absorb light and cause severe, disabling photosensitivity.

Beneficial effects of light- Photomedicine is also concerned with the beneficial effects of light. For example, phototherapy is useful for treating jaundice in premature babies, and light-based therapies can be effective in treating psoriasis. The light-based techniques are used to treat tumors and to inactivate the human immunodeficiency virus that causes AIDS and other viruses present. Phototherapies for several other diseases exist or are under development by the photomedical community.

Photoprotection. Both topical and systemic sunscreen agents prevent the acute and chronic effects of sunlight. They enable people to work outdoors and enjoy outdoor activities with reduced risk of sun-induced injury. The damage that absorbed light creates in the skin, such as the changes recognized as aging of the skin, is preventable by using new types of water- and sweat- resistant sunscreens.

Photoimmunology. Light exposure can affect the immune system. For example, irradiation of mice with ultraviolet light not only produces skin tumors at the site of exposure, but also alters the entire immune system, allowing transplantation of the tumors to areas not exposed to light. Studies of these effects may ultimately help explain the molecular basis for skin cancer in humans. This and other effects of light on the immune system are currently under active investigation in many photobiology laboratories.

Environmental Photobiology

Environmental photobiology is a new, multidisciplinary research area. It is concerned with the effects of artificial light on the human environment, the effects of sunlight on ecosystems, and human influences on the quality of sunlight reaching the earth's surface. The role of artificial light on the human environment has only begun to receive serious attention, and is an exciting challenge for the future.

Photosensitization phenomenon occurs not only in humans, as described in the section on photomedicine, but also in other organisms. For example, some plants contain potent photosensitizing chemicals. When cattle, sheep or other animals eat these plants they become light sensitive and may even die if they remain in the sunlight. Grazing animals with liver dysfunctions also become light sensitive due to the accumulation of chlorophyll metabolites that are photosensitizers. Even foodstuffs can suffer from photodamage. Some snack foods such as potato and corn chips develop an off flavor when exposed to light. This apparently results from the photooxidation of unsaturated oils that remain in the chips after cooking.

Photobiologists working with ultraviolet radiation are concerned with identifying the photochemical changes that are produced in living tissue by the absorption of ultraviolet light and determining the biochemical and physiological responses of cells to this damage.

Photochemistry

At the turn of twentieth century, photochemistry was barely developed as a science. Although many photoreactions were known due to accidental or intentional exposure of substrate to (sun) light,[2] the underlying principles were poorly understood. The "first law of photochemistry" had been recognized by Grotthus (1817) and Draper (1843), but the quantum nature of light and its consequences for photochemistry were yet to be discovered.

A little more than two decades ago a vigorous interest in the photoreactions of organic molecules began to develop. That initial interest was followed by tremendous research effort in the area of photochemical mechanism and was paralleled by remarkable and rapid technological breakthroughs in generation and control of light, especially in the area of pulsed excitation, which allow the study of dynamic processes with duration as short as 10^{-12} second. The new respectability, which resulted from the development of a mechanistic framework for photoreactions encouraged further research and early breakthroughs in exploratory photochemistry and underlying physical principles led to new diverse, yet inter-related area of research. Two types of developments, one based on techniques and one based on new theories and concepts have been vital for the growth of photochemistry. These developments are well recorded in the many fine books of photochemistry.[3-7]

Thus in the 20th century, photochemistry blossomed from a poorly defined to a highly sophisticated science, and these days it is considered a thrust area of research activity worldwide for its diverse scope in chemical and biological sciences.

Photochemical changes are responsible for biological responses to light, and can have important effects on the environment of all organisms, including humans. Understanding and control of any photobiological process requires knowledge of the underlying photochemistry, and hence photochemistry is becoming increasingly important as a tool in biological research. For example, the understanding of many complex photobiological processes can be enhanced through the preparation and study of synthetic photochemical models.[1] Photochemistry can also be used to study the spatial relationships of molecules in complex biological structures. In this approach, light is used to induce chemical bonds between adjacent molecules. Subsequent identification of these attachments indicates the spatial relationship of the molecules in the native biological structure.

Many important industrial and manufacturing processes are based on photochemistry. Photocopying, photography and photolithography are just a few examples. Also, natural and synthetic chemicals (e.g., medications, industrial chemicals, herbicides and pesticides[8]) can sometimes be altered by sunlight to produce compounds toxic to humans and other organisms or harmful to the environment.[9] For example, the action of sunlight on automobile exhaust contributes to smog.

Thus the importance of photochemical reactions to mankind is tremendous. Although the same kind of reactions are found in photochemistry as in thermochemistry, the reactivity of a molecule in its ground state and in its excited state mostly differ from each other, not only qualitatively but also quantitatively. Some examples serve to illustrate the point:

1. Thermochemically (Δ) the chloramphenicol is very stable in aqueous solution; e.g. at 25°C and pH 7 it has a half-life of more than 2 years.[10] The major cause of chloramphenicol degradation under these conditions can be attributed to the hydrolytic cleavage of the amide bond (Fig. 1.1).

In contrast, homolysis occurs photochemically ($h\nu$) at the carbon atom adjacent to the aromatic ring. During exposure to sunlight of moderate intensity ($I = 14$ W m^{-2} at 360 nm) for 45 min at room temperature, more than 80% of initial chloramphenicol (10 mg l^{-1} in 0.05 M phosphate, pH 7.0) proved to be decomposed. In addition to 20% unconverted material, 25% *p*-nitrobenzaldehyde, 15% *p*-nitrobenzoic acid and 36% *p*-nitrosobenzoic acid were found.[11] Formation of these reactive photoproducts can have toxicological implications, if only because the concentration in the aqueous humour of the eye after topical and systemic application of the drug is approximately 5-15 mg l^{-1} and 5-30 mg l^{-1} respectively[12] for some hours.

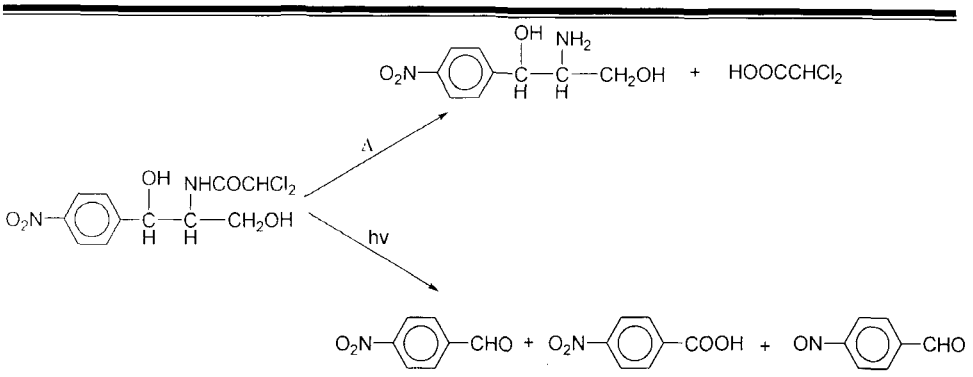


Fig. 1.1 Thermochemical (Δ) hydrolysis and photohomolysis (*hv*) of chloramphenicol in aqueous medium.

2. The tranquillizer chlordiazepoxide (1 mg ml^{-1}: pH 7) decomposes very slowly into demoxepam upon standing in. the dark at room temperature ($t_{0.5}$ = 2 h at 80°C).[13] Demoxepam is one of the main metabolites of chlordiazepoxide in man.

   When the same solution is exposed to UV radiation (λ_{max} = 350 nm: I = 20 W m^{-2}. intensity comparable to that on a sunny May day in Holland) the half-life is only 40 s. However, in contrast to the thermochemical (Δ) reaction where hydrolysis occurs, chlordiazepoxide isomerizes into an oxaziridine (Fig.1.2). This photo-isomerization (hv) into a very reactive oxaziridine has been shown to be responsible for the phototoxic and photoallergic properties of chlordiazepoxide and other imino-N-oxides.

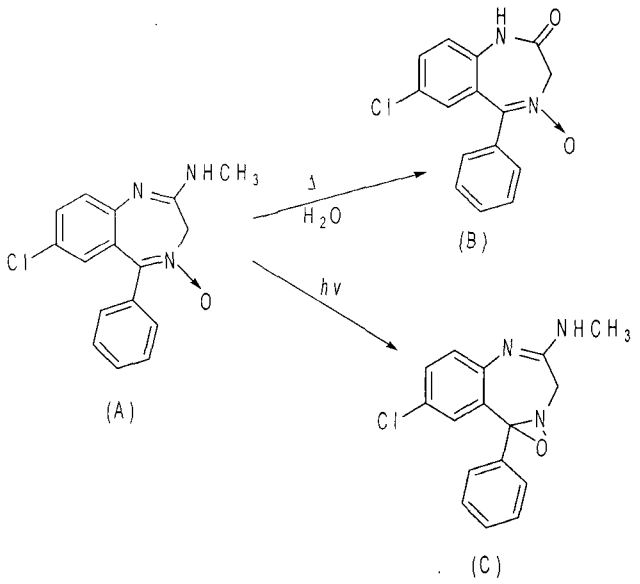


Fig. 1.2 Thermochemical (Δ) hydrolysis of chlordiazepoxide (A) into demoxepam (B) and photoisomerization (*hv*) into its oxaziridine (C).

Because a photochemical reaction only take place with molecule in an excited state, a knowledge of the formation of excited states, its kind, and the processes they undergo apart from chemical reaction is *essential to understand the photochemistry of an organic molecule or biologically active substrate such as drugs.* The electronic arrangements in molecular orbitals during electronic transition leads to singlet and triplet states differing in spin multiplicities. A state can be specified by its spin multiplicity (2S+1) (Table 1.1).

No. of unpaired Electrons	S	Multiplicity	State
0	S= 0	2S+1 = 1	singlet
1	S= 1/2	2S+1 = 2	doublet
2	S= 1	2S+1 = 3	triplet
3	S= 3/2	2S+1 = 4	quartet

Table 1.1

For any molecule there is a series of excited states obtained by different electronic transitions designated as: excited singlet state (S_1, $S_{2.....}$) and excited triplet state (T_1, T_2, $T_{3...}$). A triplet state always has a lower energy than singlet state (due to Hund's rule). Triplets are rarely obtained directly by photon excitations; they are obtained indirectly by singlets or by energy transfer.

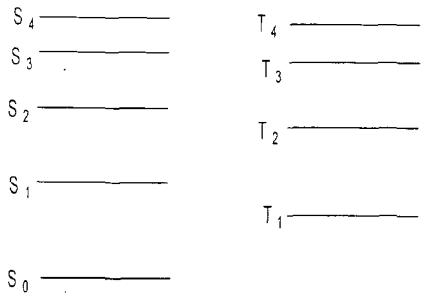


Fig. 1.3 Some electronic energy states of a normal organic molecule represented in a Jablonski diagram. Subscript 0 indicates the ground state, while the higher subscripts point to singlet (S) and triplet (T) excited states.

The other point of significance is the formation of excited states and the physical deactivation processes they can undergo apart from chemical reaction:

In the absence of surrounding molecules: Absorption of energy, UV-radiation or visible light, by a molecule only occurs in discrete quantities, photons. If a photon is absorbed then its energy corresponds to the difference in energy between the excited state involved and the ground state. During excitation of the S_0 to one of the T_n states both the orbital and the spin of the electron must change. However, absorption takes place in only

10^{-15} s and this makes such a simultaneous change of both orbital and spin of the electron involved highly improbable. An importance consequence of this is that with organic molecules, which normally have singlet ground state (S_0), absorption of light exclusively produces singlet excited states and not triplets.

The lifetime of excited singlet states other than S_1, is usually smaller than 10^{-11} s; too short for a photochemical reaction. Within this short time, molecules in higher excited singlet states tumble down to lowest vibrational level of their S_1 state. In fact the energy gap between the higher excited states and S_1 state is much smaller than that between the S_1 and the ground state S_0. This facilitates the rapid decay of highly excited states to the S_1state. The larger energy gap between the S_1 and S_0 states results in a longer lifetime of the molecule in the S_1 state: 10^{-9}-10^{-6}.

Important consequences of this are:

1. Although absorption of light is an essential condition for photochemistry, the nature of photochemical reaction is independent of the wavelength of the radiation used. This is due to the sequence:

$$h\nu : S_0 \longrightarrow S_n$$
$$\Delta : S_n \rightarrow \rightarrow \rightarrow \rightarrow S_1 \rightarrow \text{reaction}$$

2. The extinction coefficient (ε) is a measure of the efficiency with which light of a given wavelength ultimately produces the first excited singlet state. A consequence of (1) and (2) is that the rate of photoreaction is a function of ε.

From the foregoing it follows that it is a misconception to believe that light of shorter wavelength is more detrimental to chemicals than light of longer wavelengths. For example protoporphyrin is 50 times more photoreactive with visible light of 410 nm than with the same intensity of UV-radiation of 330 nm. The reason for this is that $\varepsilon_{410} \approx 50 \times \varepsilon_{330}$.

Because of the vast majority of organic molecules, light absorption ultimately leads to an S_1 state, it is important to know what kind of processes can take place from that excited state.

There are radiative and non-radiative ways to lose the excitation energy (Fig.1.4). The non-radiative processes, a and b, initially proceed without loss of energy; a high vibrational level is reached of either the S_1 or the T_1 state, followed by a rapid cascade to the lowest vibrational level. Both processes are probable because only the orbital of electron (a) or its spin (b) is changed. Process (a) is called internal conversion (IC) and (b) intersystem crossing (ISC).

The most important consequence of process (b) is that the triplet excited state, which is almost not formed upon light absorption, can often easily be obtained via the probable processes $S_1 \rightarrow T_1$ (ISC). The other non-radiative process, from the triplet T_1 to a high vibrational level of the ground state S_0 is improbable because the orbital and the spin of the excited electron should be simultaneously changed.

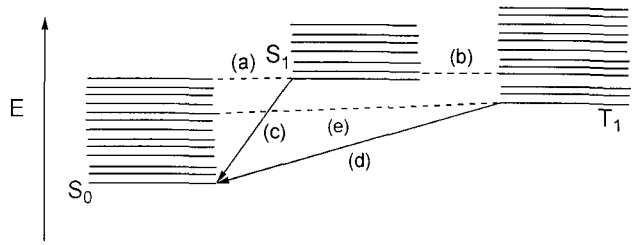


Fig. 1.4 Physical deactivation processes from the first excited state without surrounding molecules being present (electronic and vibrational levels are represented). (a) $S_1 \rightarrow S_0$ + vibrational and rotational energy; (b) $S_1 \rightarrow T_1$ + vibrational and rotational energy; (c) $S_1 \rightarrow S_0 + h\nu$ (fluorescence); (d) $T_1 \rightarrow S_0 + h\nu$ (phosphorescence); (e) $T_1 \rightarrow S_0$ + vibrational and rotational energy

Radiative processes are (c) from the S_1 and (d) from the T_1, called fluorescence and phosphorescence respectively. With (c) only the orbital and with (d) both the orbital and the spin should changed, making (c) a probable and (d) an improbable process.

The important consequences of the fact that processes (a), (b) and (c) from the S_1 state are probable and (d) and (e), from the T_1 state, are improbable processes, is that the lifetime of the molecule in the triplet excited state is far longer than that in the singlet excited state. The intrinsic lifetime, without surrounding molecules, is 10^{-3}-10 s for the T_1 state and 10^{-9}-10^{-6} s for the S_1 state. Because the intrinsic lifetime of a molecule in its T_1 state is much longer, the change of colliding with a reaction partner is far greater than when it is in the S_1 state. This is the reason that the T_1 state is so important to photochemistry.

In addition to the physical relaxation processes mentioned (Fig 1.4) competing photochemical reactions can take place in which surrounding molecule are not necessarily involved (unimolecular reaction). Thus isomerization, rearrangement and radical formation are examples of reactions that a molecule in the excited S_1 or T_1 state can undergo. Because unimolecular reactions can proceed very rapidly, the change that a molecule in its singlet excited state will undergo this kind of reaction should be taken in to account.

In the presence of surrounding molecules (in particular energy transfer).

In the presence of other molecules (X) formation of excited states and subsequent deactivation processes are essentially the same as described in the previous section. A number of processes can occur, some of which are rather trivial:

$$S_1 \text{ (or} T_1) + X \longrightarrow S_0 + X + \text{Kinetic energy}$$

$$S_1 \text{ (or} T_1) + X \longrightarrow \text{Reaction products}$$

The first process is called collisional deactivation to which, evidently, the T_1 state with its longer intrinsic lifetime is more susceptible. The same holds for the formation of reaction products. (However, one should be careful when considering the T_1 state as a prerequisite for photochemical reaction. Especially when reactants are very near, as for the complexation of drugs with bio-molecules, the S_1 state can considerably contribute to product formation.

Apart from the trivial processes mentioned, the presence of other molecules can produce another effect, called energy transfer, which complicates the photochemical activity of drugs. This involves transfer of excitation energy from an electronically excited molecule (D = Donor) to the ground state of another molecule (A = acceptor) resulting the deactivation of D and excitation of A. Excited D involves either the S_1 or T_1 state. Light absorption, followed by radiation less deactivation of excited singlet states with n>1, produces the S_1 state. The T_1 state results from intersystem crossing (ISC):

$$h\nu : D(S_0) \longrightarrow D(S_n)$$
$$\Delta : D(S_n) \rightarrow\rightarrow\rightarrow\rightarrow D(S_1)$$
$$ISC : D(S_1) \longrightarrow D(T_1)$$

Between organic molecules, of which the ground state usually is a singlet, two modes of energy transfer, (a) and (b), are possible (the arrows in parentheses represent the electron spins of the outer two electrons; subscript s or t means singlet or triplet).

$$D_s(\downarrow\uparrow) + A_s(\downarrow\uparrow) \longrightarrow D_s(\downarrow\uparrow) + A_s(\downarrow\uparrow)$$
$$D_T(\uparrow\uparrow) + A_s(\downarrow\uparrow) \longrightarrow D_s(\downarrow\uparrow) + A_T(\uparrow\uparrow)$$

Oxygen, which often plays a role in photochemical processes of bio-molecules, is a triplet in its ground state. In connection with energy transfer, route (c) in which A = O_2 is also important. Singlet has a longer lifetime. The reason is that the transition to the triplet ground state is improbable because the orbital and the spin orbital should be simultaneously changed.

$$D_T(\uparrow\uparrow) + A_T(\downarrow\downarrow) \longrightarrow D_s(\downarrow\uparrow) + A_T(\downarrow\uparrow)$$

In processes (a), (b) and (c), both D and A undergo an electronic transition. Consequently, this would mean that (a) would be the only probable process. The reason is that both with (b) and (c) simultaneous change of spin of the electrons involved in the excitation of A and in the deactivation of D also occurs. However, (b) and (c) can still occur by the virtue of exchange interaction between D and A. This means that the molecular collision or near collision between D and A should take place, such that the electron clouds of the donor and acceptor overlap. In the region of overlap, the excited electron of D and outer electron of A are indistinguishable and can exchanged without their spin being changed.

Although for route (a) to produce a collision between D and A is not necessary and the distance between the two can as much as 5 nm, the So $\rightarrow$ S_1 part of the absorption spectrum of the acceptor should partly overlap the fluorescence spectrum ($S_1 \rightarrow S_0$) of the donor: the energy difference between $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_0$ should not be too large. Because a (near) collision between donor and acceptor is a condition for the occurrence of both (b) and (c), it is understandable that a long lifetime of the excited D as a triplet will favour the occurrence of processes (b) and (c). Another condition for both (a), (b) and (c) to proceed, is that in each case the energy for deactivation for excited D exceeds that for excitation of ground state A; e.g., process (b): $E (D_T \rightarrow D_S) > E (A_S \rightarrow A_T)$.

Whether conditions of overlap of spectra, lifetime of excited state and difference in deactivation and excitation energy are fulfilled in a given case can be concluded from the study of the electronic spectra (absorption,

fluorescence and phosphorescence) of D and A. Whereas absorption spectra mostly consist of more bands, the fluorescence and phosphorescence spectra have one band only. The reason is that the higher excited singlet states, formed upon absorption, live too briefly and tumble down in a non-radiative process to the lowest vibrational level of the S_1 slate. From the latter energy level, fluorescence can take place, which brings the molecule into one of the vibrational levels of its ground state, S_0. In the same way, phosphorescence occurs from the lowest vibrational level of the T_1 state, reached from the S_1 state by ISC. As a result of the latter, both fluorescence and phosphorescence bands of a given compound are at the long wavelength side of its absorption spectrum. Further, the fluorescence spectrum ($S_1 \rightarrow S_0$) is often a mirror-image of the long wavelength absorption band ($S_0 \rightarrow S_1$).

Energy transfer from an excited donor to a ground state acceptor mostly proceeds very efficiently. This holds for the processes (b) and (c), and not only for (a) in which a collision is not even necessary (distance effect). A frequently observed situation is that the donor itself does not react during the process of photosensitization. If this is the case, the donor can repeat the transfer of absorbed energy numerous times per second and act as a catalyst. The consequence is that the donor, also called photosensitizer, can be present in a very low concentration.

Oxygen is also an ubiquitous element of our environment with a tremendous impact on animal and plant life. Atmospheric oxygen has been recognized for more then 100 years as the principal agent responsible for the deterioration of organic materials exposed to air. The parallel role of

oxygen, ***a molecule essential for many forms of life***, as a destructive (toxic) agent for living tissues has been discovered much more recently.[14] Oxygen is not only fundamentally essential for energy metabolism and respiration, but it has been implicated in many diseases and degenerative conditions. A common element in such diverse human disorders as ageing, arthritis, cancer, Lou Gehrig's disease and many others is the involvement of partially reduced forms of oxygen.

Atmospheric oxygen in its ground-state is distinctive among the gaseous elements because it is a triplet biradical, or in other words it has two unpaired electrons with parallel spins. This feature makes oxygen paramagnetic; it also makes oxygen very unlikely to participate in reactions with organic molecules unless it is "activated". According to Pauli's exclusion principle, this precludes reactions with a divalent reductant, unless this reductant also has two unpaired electrons with parallel spin opposite to that of the oxygen, which is a very rare occurrence. Hence, oxygen is usually non-reactive to organic molecules, which have paired electrons with opposite spins. Activation of oxygen may occur by two different mechanisms: absorption of sufficient energy to reverse the spin on one of the unpaired electrons, or monovalent reduction. If triplet oxygen absorbs sufficient energy to reverse the spin of one of its unpaired electrons, it will form the singlet state, in which the two electrons have opposite spins. This activation overcomes the spin restriction and singlet oxygen can consequently participate in reactions involving the simultaneous transfer of two electrons (divalent reduction). Since paired electrons are common in

organic molecules, singlet oxygen, a metastable higher energy state molecular oxygen species, is much more reactive towards organic molecules than its triplet counterpart.

Singlet oxygen a potential product of photochemical reactions of many compounds is a damaging agent to all living organisms and so it also places a very significant role in plant metabolism.[15] Photosensitizing reactions among secondary plant products is a wide spread phenomenon involving ultra-violet as well as visible light. Secondary plant substrates of diverse biogenetic origin are capable of the photogeneration of singlet oxygen and they act as quenchers of singlet oxygen, suggesting the wide spread use of these potent toxic and as protective and defensive agents. Oxygen plays an important role in aging, damage to materials in the environment, cellular pathology (for example, the damage following stroke or heart attack) and many other areas. The details of the chemical reactions underlying these processes are poorly understood.

Singlet oxygen ($^1O_2$) was first observed in 1924 and then defined as a more reactive form of oxygen. In 1931, Kautsky[16] first proposed that $^1O_2$ might be a reaction intermediate in dye-sensitized photooxygenation. Following this suggestion, many studies have showed that $^1O_2$ is an important intermediate species in the detrimental oxidation of biomolecules. Singlet oxygen is very toxic to organisms because it reacts with important biological molecules such as unsaturated lipids,[17-19] oxidizable amino acids,[20-22] and nucleic acids.[23,24] particularly guanosine derivatives.[25] The resulting reactions cause

destruction of membranes, enzyme inactivation, and mutations, all of which can lead to cell death.

Since oxygen is ubiquitous and efficiently quenches electronically excited states, $^1O_2$ is likely to be formed following irradiation in countless situations and involved in various chemical and biological processes as well as in several disease processes. Reactive intermediates include[26] singlet oxygen ($^1O_2$), a metastable excited state of molecular oxygen), superoxide ion, and other oxygen species. $^1O_2$ O_2 $HO^{\bullet}$ $HO^{\bullet}_2$ $RO^{\bullet}_2$

Electronic structure and the lifetime of singlet oxygen

Molecular orbital theory can explain the electronic structure of molecular oxygen. The lowest electronic state of oxygen is a triplet ground state ($^3\Sigma g^-$) with two unpaired electrons distributed in the highest occupied orbitals. Rearrangement of the electron spins within these two degenerate orbitals results in two possible singlet excited states. The $^1\Delta g$ state has an energy only 23 kcal above that of the ground state; both electrons are paired in a single orbital, leaving the other vacant. This state might be expected to undergo two-electron reactions. The higher singlet state ($^1\Sigma g^-$) comes from the spin pairing electrons in different orbitals and might be expected to undergo one-electron free-radical reactions. Because there are no unpaired electrons, neither $^1\Delta g$ nor $^1\Sigma g^+$ are radicals. In both forms of $^1O_2$, the spin restriction is removed so that the oxidizing ability is greatly increased. The $^1\Sigma g^-$ state has a much shorter lifetime than $^1\Delta g$ state because $^1\Sigma g^+$ is more reactive than the $^1\Delta g$ form, it decays to $^1\Delta g$ state before a chemical reactions can occur.

Generation of singlet oxygen

Both physical and chemical methods are used to generate singlet oxygen.

Physical methods: These are based on photosensitization and proceed by two different processes called Type I and Type II[27] (Fig.1.5). Usually the direct radical mediated reactions are called as Type I reactions where as singlet oxygen mediated reactions are considered as Type II reactions.

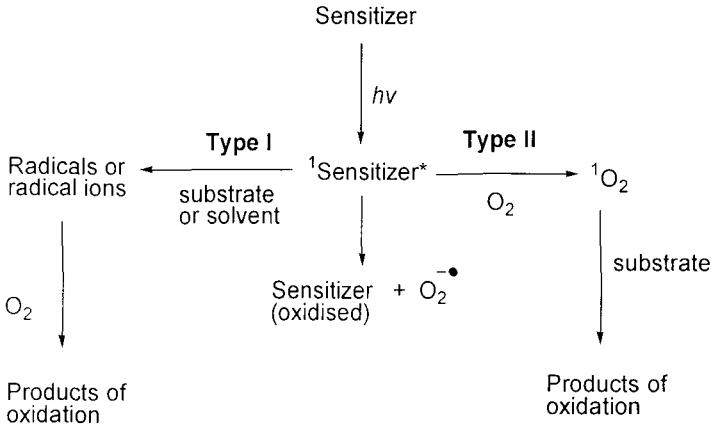


Fig. 1.5

The most common method of generating singlet oxygen in organic solvents has been the use of dyes in solution with the reagents.[28] The irradiation of the solution excites the dyes to a higher singlet state, which then undergoes intersystem crossing to the longer lived triplet state (Fig. 1.6). This state transfers its energy to form singlet oxygen from the triplet ground state.

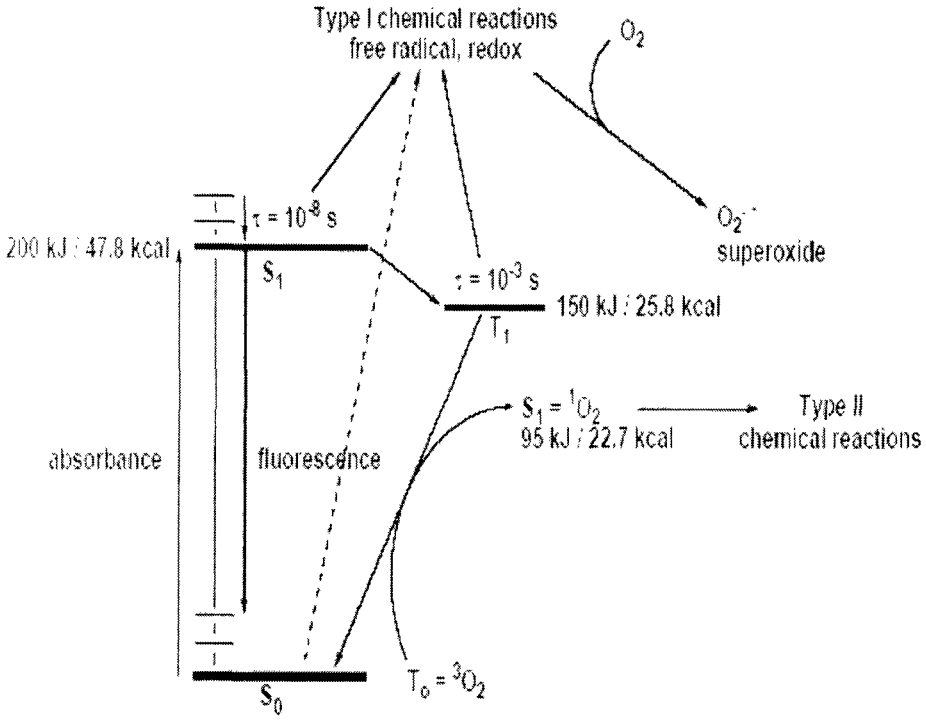


Fig. 1.6. Photosensitizer mechanism

Commonly used dyes include Rose bengal (**1**), methylene blue (**2**) and tetraphenylporphyrin (**3**) shown in Figure 1.7. The choice of dye is based on high absorbance in the visible region, the half-life and energy of the triplet state, and the efficiency at exciting oxygen in the reaction solvent. Although the dyes are catalytic, they are slowly degraded by singlet oxygen in a photobleaching process and longer photooxygenation require higher dye loading to be efficient. As a result, this technique is not amenable to large-scale reactions and alternative photooxidations methods have been investigated.

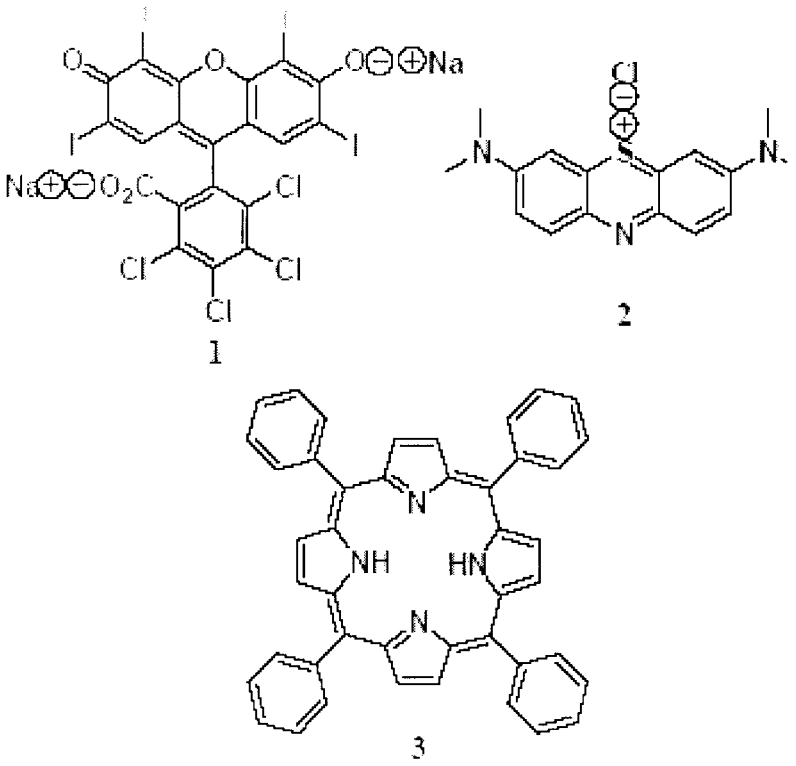


Fig. 1.7 Dye structures

The useful photosensitizers[29] for singlet oxygenation are given in table...

Sensitizer	Triplet energy	
	KJ mol^{-1}	Kcal mol^{-1}
Methylene blue	142.2-142.3	33.5
Tetraphenyl porphyrin	142.3	34.0
Hematoporphyrin	155.7	37.2
Rose bengal	164.1-176.6	39.2-42.2
Eosin	180.8-192.5	43.2-46.0
Benzophenone	287.2-289.2	68.6-69.1

Table 1.2

Fréchet has reported the use of dendrimers as catalysts for singlet oxygen reactions.[30] The dendrimer structure shown in Figure 1.8 contains a hydrophobic core with a photosensitizing moiety. Nonpolar reagents diffuse into the reaction core while polar products diffuse back into solution. This method would be expected to work best when there is a large polarity difference between product and reactant. Benaglia and coworkers have fixed 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin onto polyethylene glycol resins to generate a catalyst that is soluble in methylene chloride.[31] Upon reaction completion, the catalyst may be precipitated with the addition of diethyl ether, filtered and recycled up to six times without activity loss. Polymer-bound photosensitizers show higher stability toward photobleaching and minimize dye decomposition and solution contamination. Free and polymer-bound dyes exhibit the same product selectivity and comparable yields.

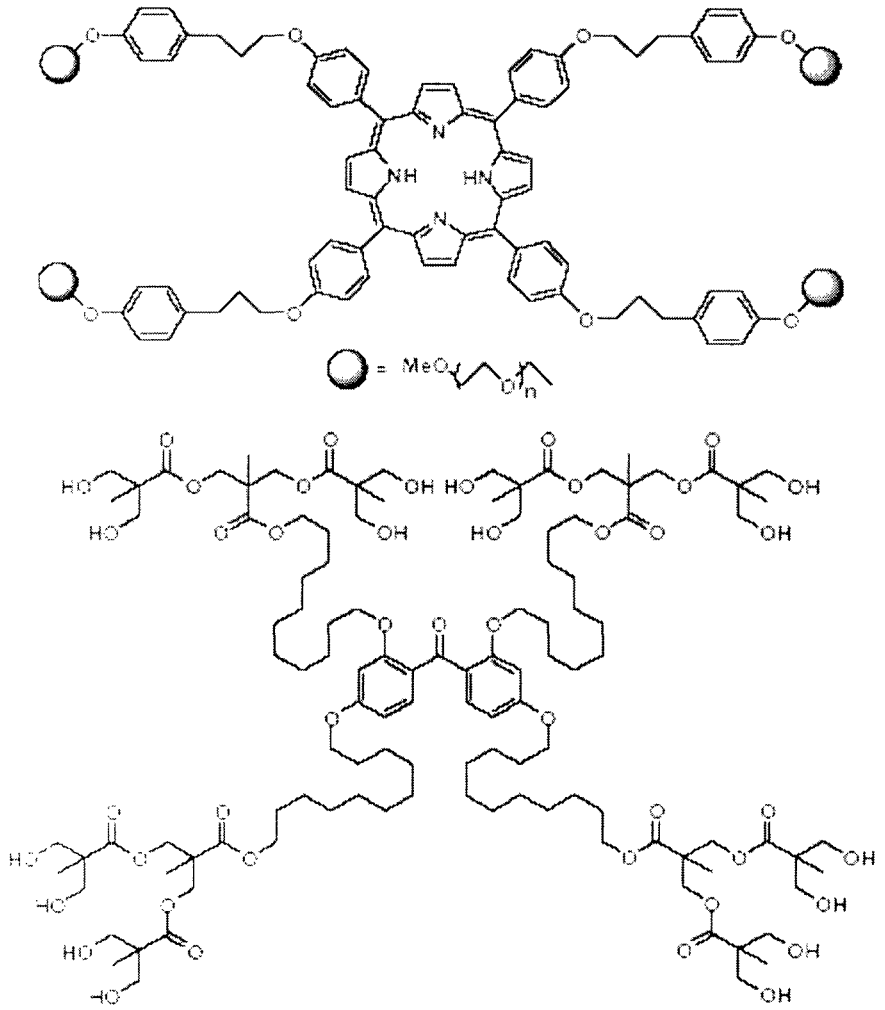


Fig. 1.8. Polymer supported dye and dendrimer for photooxygenation.

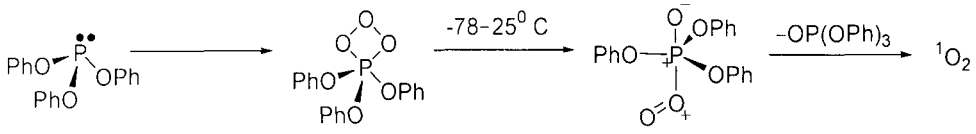
Alternatively, singlet oxygen can be "stored" for several weeks in the form of calcium peroxide diperoxohydrate ($CaO_2.2H_2O_2$) at -80 ^{o}C.[32] ($CaO_2.2H_2O_2$) can be made by reacting H_2O_2 with $CaCl_2$ or $CaO_2.8H_2O$. The yield of $^1O_2$ from the calcium salt upon thermolysis at 50° C is observed to be 25 % by titration with a reactive cyclic diene, α-terpinene. This method is most efficient for compounds that readily undergo photooxygenation.

However, when physical and chemical quenching becomes important, longer reaction times and higher calcium peroxide diperoxohydrate loading are required.

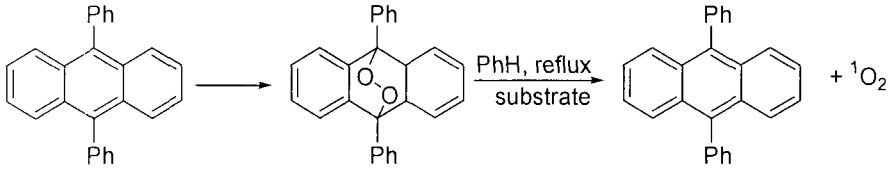
Chemical methods

Some chemical methods[33,34] used to generate $^1O_2$ are:

a) Murray Method: thermal decomposition of ozone-phosphite adducts



b) Thermal decomposition of aromatic endoperoxides



c) Decomposition of hydrogen peroxide with bleach

$$H_2O_2 + OCl^- \longrightarrow HO_2^- + HOCl \longrightarrow HO^- + HOOCl \longrightarrow H_2O + ClOO^-$$

$$ClOO^- \longrightarrow Cl^- + {}^1O_2$$

This is one of the biologically significant reactions because OCl^- can be formed by the enzyme myeloperoxidase during phagocytosis.[35]

Generation of Singlet oxygen in biological systems:

Singlet oxygen in biological systems can be generated in two different ways;[36] by 'light reaction' due to photoexcitation and by 'dark reaction' due to chemi-excitation. A major route for the process is by the type II

photosensitization reaction resulting in an energy transfer from triplet state of photosensitizer to ground state molecular oxygen.

Many cellular constituents such as flavins, porphyrins, cytochromes etc. have the ability to generate $^1O_2$ under illumination. The dark reactions that generate $^1O_2$ include enzymatic reaction catalyzed by dioxygenases, lactoperoxidases, myeloperoxidases, cytochromes, tryptophan, pyrrolase and lipooxygenases. There are evidences for $^1O_2$ production obtained using a number of purified enzyme system. Metabolic generation of $^1O_2$ has been shown to occur in stimulated neutrophils. Some studies show that $^1O_2$ can be derived from spontaneous rather than enzymatic decomposition of super oxide. Hence superoxide dismutase indirectly protects from $^1O_2$. This species has also been found to be generated from other reactions of biological relevance such as lipid peroxidation and reaction of hydroperoxides with peroxynitrite.

The formation of $^1O_2$ by these mechanisms is likely to be increased under the influence of certain xenobiotics capable of inducing oxidative stress. In the mammalian tissues, one of the main candidates for the production of $^1O_2$ is activated polymorphonuclear leukocytes. The primary function of these cells is to destroy invading microbes. In response to such stimuli, the cells generate superoxide, hydrogen peroxide and hypohalous acid during a process known as 'respiratory burst'. Fairly large and toxic quantities of $^1O_2$ are produced during respiratory burst via reactions catalyzed by lysosomal myeloperoxidase. The generation of $^1O_2$ by polymorphonuclear neutrophils or myeloperoxidase during bactericidal action, photosensitization and during lactoperoxidase activity was proved. This provided evidence showing

microbactericidal activity is related to $^1O_2$ generation. Human saliva, in presence of low amounts of hydrogen peroxide can also generate $^1O_2$.

Generation in Chloroplast:

The condition which favour $^1O_2$ production, i.e., triplet excited molecules and ubiquitous oxygen, are likely to be found with in the active chloroplast. The close proximity of excited chlorophyll and an oxygen evolving system is thus potentially hazardous as is also an active electron transport system with the potential for electron donation to oxygen to yield superoxide anion. Superoxide is probably dismutated via a linked series of enzymes involving superoxide dismutase, ascorbate peroxidase and glutathione reductase.[37]

When a chlorophyll molecule absorbs a quantum of light energy it enters in excited singlet state. Singlet chlorophyll has a lifetime of approximately 10^{-8} sec. The triplet state is not only potentially damaging itself in a type I reactions but also by virtue of triplet-triplet interaction with ground state oxygen it may generate $^1O_2$. Isolated chloroplast thylakoid membranes when incubated in absence of electron acceptors and thus likely to favour $^1O_2$ generation, have been shown to undergo pigment and lipid breakdown.[38-40] These reactions were enhanced by conditions, which promoted $^1O_2$ generation and lifetime, such as high light and oxygen.

$^1O_2$ generation in stress conditions :

A number of conditions may promote photodestruction in plants and these could involve generation and action of $^1O_2$ e.g. chlorophyll formation under high light conditions,[41] during the incubation of leaves in the absence of carbon dioxide,[42] under chilling conditions[43] and in presence of photosynthetic electron transport inhibitor herbicides.[44] Approximately half

of all currently utilized herbicides operate by inhibiting photosynthetic electron flow[45] and the inhibition of electron flow promotes the generation of ^{3}Chl and hence $^1O_2$.

Other plant products are also reported having potential to generate $^1O_2$ e.g. hypericin. Isolated from the glandular trichomes of the calyx of *Hypericium hirsutum*, is capable of generation of $^1O_2$.[46] Furanocoumarins are also capable of photodynamic reactions involving the activation of oxygen. The photogeneration of $^1O_2$ by furanocoumarins have now been thoroughly documented and indicates that they may be responsible for the direct disruption of membranes and enzymatic deactivation.[47]

Detection of singlet oxygen

The techniques of $^1O_2$ detection and measurement depend on the generating system. Some of the prevalent methods are listed below. Moreover the search for more sensitive and accurate procedure continues.

1. Scavengers: Scavengers can inhibit a reaction dependent of $^1O_2$.[48] For example, azide acting as a physical scavenger reacts with $^1O_2$ to give a reactive azide radical, $N_3^- + {}^1O_2 \rightarrow N_3\bullet + O_2$. Other scavengers including DABCO, carotene, ascorbate, thiols and histidine act as chemical scavengers.
2. D_2O: D_2O can be used to detect $^1O_2$ presence because the lifetime of $^1O_2$ is 10- fold longer in D_2O than in H_2O.[49] So, if a reaction in aqueous solution is dependant on singlet oxygen, carrying it out in D_2O instead should greatly potentate the reaction.

3. Luminescence: As $^1O_2$ decays back to the ground state, some of the energy is emitted as light. The light from singlet oxygen appears in the infrared as 1268 nm.[50] O_2 ($1\Delta g$) → O_2 ($^3\Sigma$- g) + hν(1268 nm)

Other detection methods include ESR, calorimetry, photo-ionization and mass spectrometry.

Chemical reactions of singlet oxygen

Singlet oxygen is an electrophilic species and isoelectronic with ethylene. Functionally $^1O_2$ seems to behave like an analogue of ethylene. Indeed, each of the three characteristic (fig.1.9 and 1.9a) modes[51,52] in which $^1O_2$ adds to olefins finds precedent in one of the reaction pathways of ethylene.

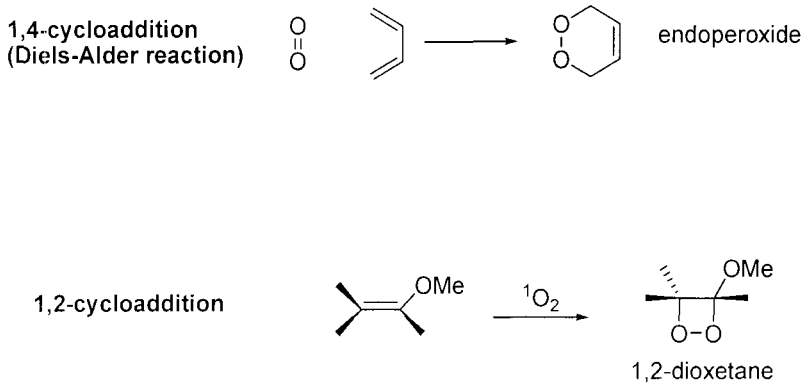


Fig. 1.9

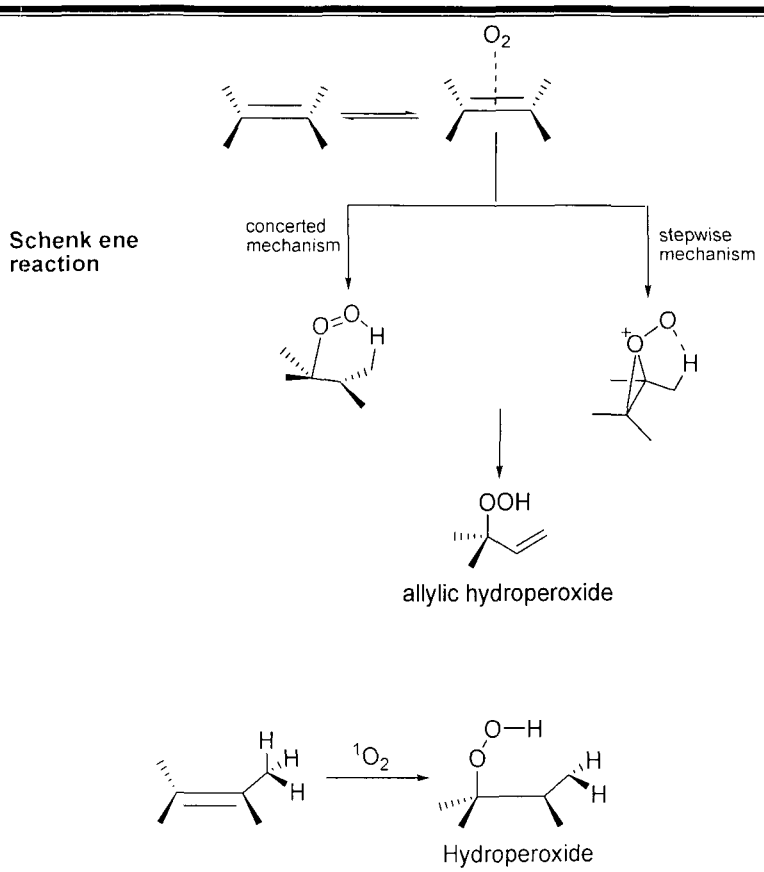


Fig. 1.9a

The addition of $^1O_2$ to dienes generating endoperoxides may be viewed as Diels-Alder reaction with $^1O_2$ as dienophile. In the $^1O_2$ "ene" reaction, olefins containing allylic hydrogens are oxidized to corresponding allylic hydroperoxides accompanied by a 1,2-shift of the double bond. In 1,2-cycloaddition, the $^1O_2$ oxygenation of certain C=C double bonds to yield dioxetanes is analogous to the production of cyclobutanes by a 2s + 2a) cycloaddition of two ethylenes.

Quenching of singlet oxygen

The quenching of singlet oxygen involves the deactivation of the excited state of molecule. Deactivation can be accomplished by either physical or

chemical quenching. Physical quenching leads only to the deactivation of singlet oxygen to its ground state with no oxygen consumption or product formation. In chemical quenching, by contrast, singlet oxygen reacts with quencher R to produce RO_2. Two major mechanisms of $^1O_2$ quenching are known, namely energy transfer and charge transfer quenching.

1. *Energy transfer quenching*

This mechanism of quenching is the reverse of reaction by which singlet oxygen is formed.[35] It involves formation of triplet quencher and ground state oxygen ($^1O_2 + Q \rightarrow {}^3O_2 + {}^3Q$).

2. *Charge transfer quenching*

This mechanism involves the interaction between the electron-deficient $^1O_2$ molecule and electron donors to form a charge transfer complex.[35] Intersystem crossing restrictions are relaxed in the complex which can then dissociate into donor and ground state oxygen.

Evolution of life is the consequence of continual response to an ever-changing environment. In the evolution only those organisms survive which can respond adequately. Every living being is forced to respond to the pressure of environment and, as a result of this, it is shaped by these responses. Thus, responses from an integrated part of an organism, in other word each animal and plant is a reflection of its environment. A delicate balance exists between the organisms and their environment. Interfering in this balance poses new problems leading to new responses; the latter may be damaging or profitable for the functioning of an organism.

Light and oxygen are ubiquitous element of our environment with a tremendous impact on life. The human body is exposed to wide array of

xenobiotics in one's lifetime, from food components to environmental toxins to pharmaceuticals and pesticides. The treatment of disease requires occasionally the use of either systemic or topical medication during certain period of time and now a days drugs have become an integral part of human life. The photobiological effects of drug, as pronounced in its photostability, photochemistry, phototoxicity and photodynamic therapy have become highly significant. Pharmaceuticals and personal care products are a class of chemicals that are continuously released into the environment through human activities.[53]One potential loss process for pharmaceuticals and personal care products is photodegradation. The potential environmental impact of these chemicals should be detected by their persistence in the environment and the biological activity of any degradation products.

The use of pest control chemicals to protect the crops from damage is unavoidable; as we need the maximum yield possible to meet our sharply increasing demand of food grains. As we know, all pesticidal molecules after application are exposed to various biotic and abiotic components of the environment and are thus subjected to various physical, chemical and biological transformation processes. It has been demonstrated over the past few years that photochemical reactions play a key role in the environmental degradation or detoxification of various anthropogenic chemicals including pesticides that contain organic chromophores or metal organic complexes capable of absorbing light energy directly.[8] Indirect photolysis or photosensitization is important especially for the pesticidal molecules having no chromophoric groups. Sensitization processes may be catalytic in

nature when the chromophore in the sensitizer molecule is regenerated by the processes of energy transfer and cyclic redox reaction.[54]

It is therefore important to study the photoreactivity of all common chemicals that may be exposed to sunlight. In the present study emphasis has been placed on drugs and biologically active plant metabolites.

The molecular mechanism of biological photosensitization by drugs is receiving increasing attention. The interest in this subject is promoted by the serious toxic reaction (mainly disease of epidermis and dermis) produced by many pharmacologically important chemicals under sunlight irradiation. Photoallergic effects, generally associated to cell mediated immunity response, and photomutagenic effects are also of current concern. Intermediates and stable photoproducts generated upon light absorption by the drugs interact with the cell components and lead to cell degeneration or death. Control of the drug photoreactivity in practical situations and preparation of protective strategies against the light-induced damage requires the understanding of the structural and environmental factors determining the photoreactivity of the various classes of drugs and of their major photoproducts, the identification of the transient species formed by photoexcitation and the study of the reactivity of these transients with the biological substrates. Information on the location and the mode of interaction of the drugs in the biological environment is also a key point.

Photobiology studies the interaction of light with living organisms. This application encompasses a number of areas including environmental photobiology, photomedicine, and UV photobiology, which is the study of the effects of ultraviolet radiation on plants, animals and humans. The

interest in human photobiology is currently growing for a number of reasons. First, sunlight is beneficial to health but overexposure as a result of increased outdoor activities can cause skin cancer[55] and accelerated aging of vital organ.[56] Further, artificial light sources, such as fluorescent tubes and the new and increasingly popular high intensity lamps are now used world wide for lighting of large public shopping areas, factory-buildings, offices and private houses. As a large part of population spends much of its time in these artificially illuminated places, in which light intensity and spectral distribution differ considerably from those found outdoors, there is a concern about the consequences for human health. Thus, it is not only because of lamps used for sunbathing that the role of artificial light in human health and diseases has begun to receive serious attention.[57] Second, spectral distribution and intensity of daylight will change if ozone layer in the stratosphere, which filters out much of the short wavelength UV radiation,[58] is deteriorated by gases from, e.g., spray cans and supersonic aircraft. A change in the spectral distribution and intensity of daylight is expected to have serious consequences for human health. Third, drugs can also interfere in the balance between light and the human body. The effects may be beneficial and thus the combination of light and drug can be used as therapeutics. Often however, the effects are unintended and uncontrolled and damage of organ functions occurs.

Spectral composition of sunlight; Subdivision of the UV radiation:[59]

The discovery of UV radiation and its effects on living organism was a gradual process that involved contribution from chemists, physicists and biologists. The cumulative evidence to the data indicates that UV radiation

has both beneficial and harmful effects depending on the type of the organism, wavelength of radiation and irradiation dose. Extraterrestrial solar radiation (200-800 nm) is essentially continuous, lacking only certain narrow wavelength bands because of the absorption by the sun's atmosphere. At midday, maximal intensity is in the region of 450-500 nm. Although there is little change in the spectral composition of sunlight as such, the intensity as a function of wavelength to which man is exposed can vary greatly depending, for example, on season, distance from the equator and height above the sea level.

The subdivision of ultraviolet radiation (UVR) into three regions[60,61] dates back to early observations by photodermatologists. UVA (320-400 nm) was considered as innoxious and responsible for pigmentation, UVB (290-320 nm) as a cause of sunburn and photocarcinogenicity and UVC (200-290 nm) as radiation, which, although photobiologically active because of its mutagenic and antimicrobial activity, is not relevant to human health provided that it is effectively absorbed by the ozone layer.

More recent research has modified this picture; e.g., both UVB and a part of UVA are carcinogenic. More photobiological effects have now been discovered which are important for the functioning of the human body and whose action spectra do not fit one of these UV-regions, e.g., influence of the UV radiation on the immune system.[62] That UVC would not be relevant to human photobiology because of the above mentioned reason is a conclusion often drawn but incorrect because it is based upon a misconception of the process of light absorption. It follows fro equations describing radiation absorption, that a certain portion is always transmitted.

Whether a small amount of the UVC transmitted by the ozone layer is important or not, should be assessed from its photobiological effects. In this connection, it is of interest to know the essential biomacromolecules have their maximal absorption in the UVC region, e.g., proteins at *c.* 280 nm and DNA at *c.* 260 nm (for comparison, current knowledge of human photobiology would not validate the conclusion that UVR is less important than visible light because UVR is only a few per cent of the electromagnetic radiation of (200-800 nm). Although as shown above, the subdivision into UVA, UVB and UVC has limited value and is inevitably dated, it is frequently used in the literature.

Phototoxic versus phototherapeutic Xenobiotics (Drugs):

Absorption of light by an endogenous compound or a xenobiotic in the skin or eyes can provoke a biological effect as a result of the following molecular processes. As far as the molecular processes underlying the occurrence of photobiological effects are concerned, there is no essential difference between the phototherapeutics and phototoxicons as a group; the same processes, a, b and c occur. One speaks of a phototherapeutic if the photoreaction is intentional and controlled. The later concerns not only the site in the body where it occurs but also the dose of the drug and the light (wavelength region, intensity and exposure time) applied.

With both phototherapeutics and phototoxicons, the sequence of events, this eventually leads to the biological effects, starts with the absorption of a photon of UV-radiation or visible light. In a biological system a photoexcited drug or other xenobiotic can undergo a number of primary

reactions (Fig. 1.10). The detailed photobiological effects of an excited drug are indicated in Fig. 1.11.

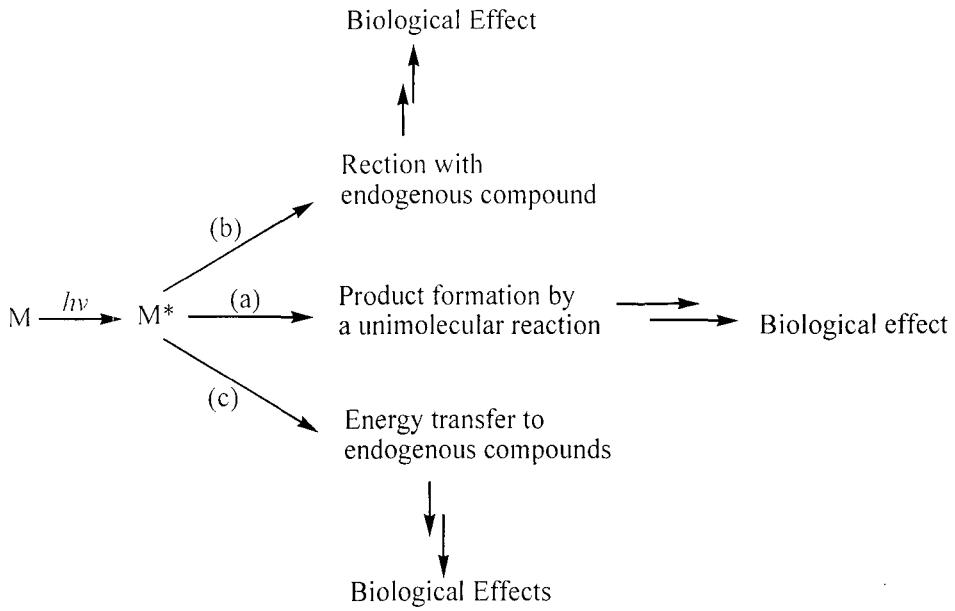


Fig. 1.10

(a) *Photochemical reaction of the compound as such*, a unimolecular reaction, e.g., rearrangement, isomerization or decomposition. The reaction products or their metabolites can display their biological activity by interaction with a receptor. At present there are no reports of phototherapeutic or phototoxic compounds acting in this way. Yet this can be considered as a possibility; certainly if one takes into account that that interaction of a photometabolite with a receptor is an essential part of a normal endogenous photobiological processes in man. This process concerns the UVB induced rearrangement of 7- dehydrocholesterol into previtamin D_3 and subsequent steps. The vitamin D process is also

important to research of the photobiological activity of the drugs, because it demonstrates that in the light-exposed skin, compounds can be formed in an extremely low concentration, which exerts their biological activity in parts of the body far away from the skin.

Unstable photoproducts can react with endogenous molecules resulting in a biological effect. If the half-life of such a photoproduct is long, it can react in inner organs after having been formed in the skin. For example, phototoxic imino –N-oxides isomerizes in the UVA exposed skin into an oxaziridine.[63] The latter can irreversibly bind to biomacromolecules.[64]

Decomposition can also involve formation of radicals,[65] which, whether or not coupled to oxygen, can damage biomacromolecule. For instance chlorpromazine photodecompose with UVA into the phenothiazine radical, this, in vivo, covalently binds to lipids and to proteins.[66]

(b) *Photoreaction with endogenous molecules*. An example of this is the irreversible binding of photoexcited psoralens to DNA as a result of UVA absorption in the skin. What is considered to play an important role in the so-called PUVA-therapy.[67-70]

Photoexcited M can also abstract an electron or an H-atom for example; endogenous glutathione and so formed M-derived radicals can damage biomacromolecules such as DNA, proteins and lipids as constituents of membranes.

(c) *Energy transfer to endogenous compounds*. This is exemplified by singlet oxygen formation from $^3O_2$, which is supposed to play an important role in dye /visible light therapy of cancer.[71-73] Phototoxic compounds of quite different molecular structure can also produce

singlet oxygen by energy transfer, e.g., phenylpropionic acid derivatives used as anti-inflammatory drugs and tetracyclines.

Each of the essential bio(macro)molecules can be damaged by primary photoreactions (a), (b) and (c) fig. 1.10. Damage to DNA can lead to cell death but also to mutation. If DNA repair enzymes (protein) are also inactivated for instance by a simultaneously occurring primary photoreactions, mutation can be transferred and tumor formation can result. Photoreaction with proteins, or with lipids as constituents of membranes, can lead to cell death, but it can also trigger an immune response, which eventually can lead to allergy. In addition to biomacromolecules such as DNA, proteins and membrane constituents, small endogenous molecules, such as glutathione, which is important to the cellular defense, are targets to primary photoreactions.

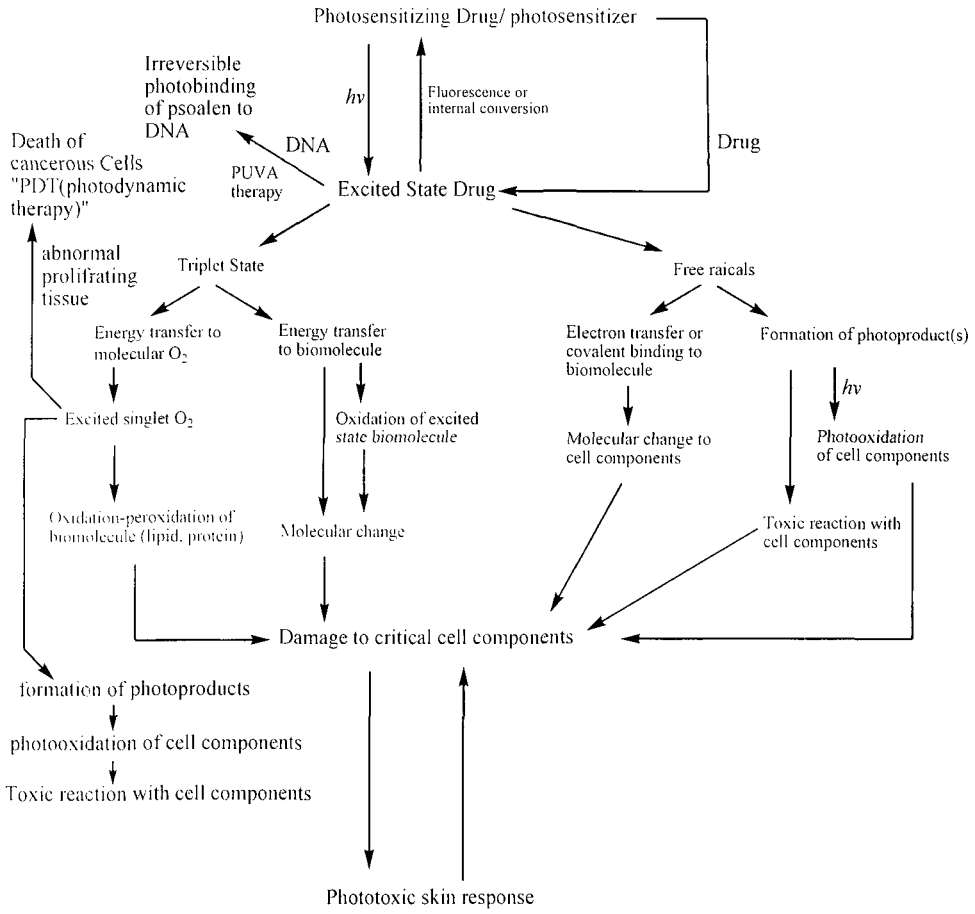

Photosensitizing Drug/ photosensitizer
Irreversible photobinding of psoalen to DNA
hv
Fluorescence or internal conversion
Death of cancerous Cells "PDT(photodynamic therapy)"
DNA
PUVA therapy
Drug
Excited State Drug
abnormal prolifrating tissue
Triplet State
Free raicals
Energy transfer to molecular O2
Energy transfer to biomolecule
Electron transfer or covalent binding to biomolecule
Formation of photoproduct(s)
hv
Excited singlet O2
Oxidation of excited state biomolecule
Molecular change to cell components
Photooxidation of cell components
Oxidation-peroxidation of biomolecule (lipid, protein)
Molecular change
Toxic reaction with cell components
Damage to critical cell components
formation of photoproducts
photooxidation of cell components
Toxic reaction with cell components
Phototoxic skin response

Fig. 1.11

The biological effects which can be observed with a given phototherapeutic or phototoxicon depends on a variety of factors, such as:

(1) The extent to which each of the primary photoreactions a, b and c occurs.

(2) The extent to which each of the essential bio(macro)molecules is damaged.

(3) The bioavailability of the given photoactive compounds and its metabolites, not only in the organs exposed to light but also at cellular level.

For example, singlet molecular oxygen is a mutagenic species. However, although dyes are efficient singlet oxygen producers by energy transfer (c), mutagenesis is not an expected problem with the dye/visible light therapy.[71-73] The fact is that the dyes commonly used in this therapy accumulate in the lipid material of the membranes and remain outside the cellular nucleus.

A comparable situation was found with furocoumarins: photobinding to DNA got by far the most attention. However, it has been demonstrated that 8-methoxypsoralen, frequently used in PUVA therapy, photobinds *in vivo* not only to DNA but to a high extent also to proteins and lipid material of the epidermal cells.[74,75]

Xenobiotics are extensively used in, for example, drugs, cosmetics, food and agricultural chemicals. Although very useful and almost indispensable they can produce adverse biological (toxic) effects. *It is commonly assumed that phototoxicity of drugs and other xenobiotics remains restricted to the organs exposed to light, namely the skin and eyes. However, there are natural*

photobiological processes in man by which the eventual effects occurs in a part of the body far from the sunlight exposed organ in which photoexcitation of an endogenous compound took place.

Insight into the molecular processes which a given phototoxicon undergo *in vivo* after absorption of UV or visible light, is a prerequisite for the identification of that part of molecular structure which causes the unwanted photobiological effect. Only after this identification will it be possible to alter the molecular structure of the xenobiotic in such a way that the phototoxicity diminishes whereas desired properties remain conserved. It has been indicated by Beijersbergen van Henegouwen[59] that this research aim can be achieved by combining data from *in vitro* and *in vivo* investigations. The *in vivo* system is too complicated and without continuous help from in vitro research, the investigation of this cannot provide much insight. Photoreactivity of the phototoxic xenobiotic and structural analogues should be studied:

1. *In vitro*, weather or not in the presence of essential bio (macro) molecules.
2. In microbiological test systems (e.g., bacteria and yeast) and in cells, whether or not in culture.
3. In experimental animals or human volunteers.

Both with (1), (2) and (3), attention should be paid to the identification of photoproducts and to the possibility that photobinding to and damage of biomacromolecules has occurred. These data can provide insight into the formation of reactive intermediates and into the reaction mechanism occurring in vivo. The in

vivo system is too complicated and without continuous help from in vitro research, the investigation of this can not provide much insight. For this reason, the above mentioned research lines (1), (2) and (3) should be performed in continuous interaction with each other.

The photochemical studies in the present work is an attempt to address in vitro photoreactivity of drugs and biologically active plant metabolites. On the basis of photochemical principles the many photochemical reactions now known have been rationalized. This is shown in many fine books of photochemistry,[3-7] which demonstrate both the dramatic development of this science in last decades and high degree of rationalization that has been reached. The photoreactions of drugs[76] obviously can be discussed in the same way. It is therefore generally possible to predict the photochemical behavior of a new drug, as of any other molecule, or at least to point out the most likely alternatives. More exactly, as it has been pointed out by Grenhill,[77] it is possible to indicate some molecular features that are likely to make a molecule liable to photodecomposition, even if it is difficult to predict the exact photochemical behaviour of a specific molecule.

At any rate, several chemical functions are expected to introduce photoreactivity (Scheme 1.1). These are:

(a) The carbonyl group. This behaves as an electrophilic radical in the $n\pi^*$ excited state. Typical reactions are reduction via intermolecular hydrogen abstraction and fragmentation either via α-cleavage (Norrish Type I) or via intramolecular γ-hydrogen abstraction followed by C_α-C_β Cleavage (Norrish Type II).

(b) The nitroaromatic group, also behaving as a radical, and undergoing intermolecular hydrogen abstraction or rearrangement to nitrite ester.

(c) The N-oxide function. This rearranges easily to an oxaziridine and the final products often result from further reaction of this intermediate.

(d) The C=C double bond, liable to E/Z isomerization as well as to oxidation.

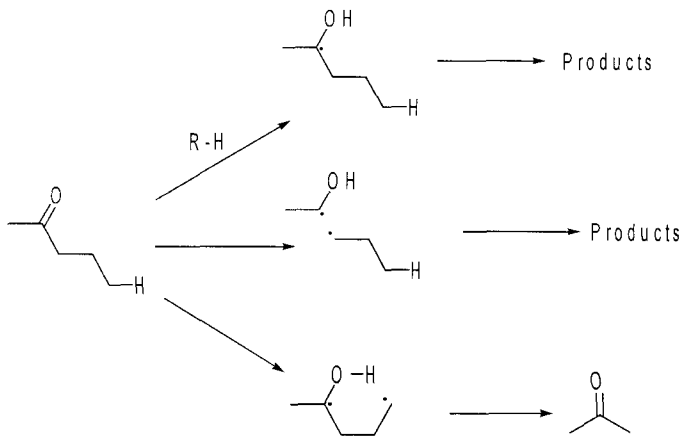
(e) The aryl chloride, liable to homolytic and/or to heterolytic dechlorination.

(f) Products containing a weak C-H bond, e.g., at a benzylic position or α to an amine nitrogen. These compounds often undergo photoinduced *fragmentation via hydrogen transfer or electron proton transfer.*

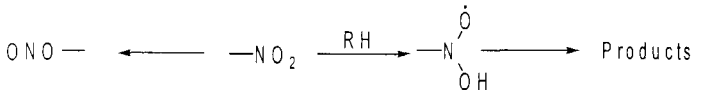
(g) Sulphides, alkenes, polyenes and phenols, are highly reactive with singlet oxygen, formed through photosensitization from the relatively harmless ground state oxygen.

Such functions are present in a very large fraction, if not the majority, of commonly used drugs. Thus many drug substances, and possibly most of them, are expected to react when absorbing light.

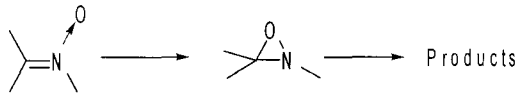
(a)


OH
Products
R-H
OH
Products
O
H
O–H
O

(b)


ONO—
—NO2
RH
O
N
OH
Products

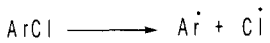
(c)


O
N
O
N
Products

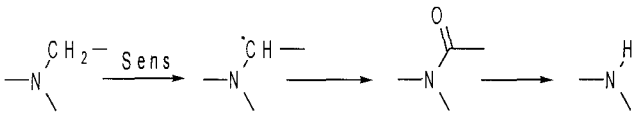
(d)

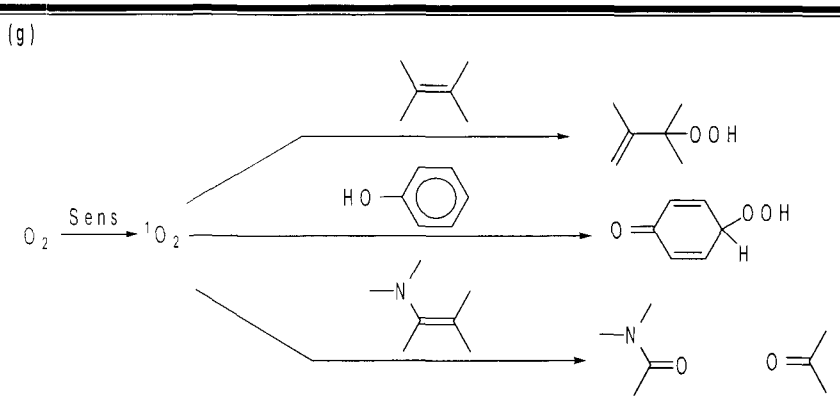

R
R''
R
R''

(e)


ArCl
Ar + Cl

(f)


CH2—
Sens
CH—
O
N
H



Scheme 1.1

Thus photochemical studies on drugs and promising drug molecules is an vital area of importance in current medicinal chemistry, for establishing a relation to its phototoxicity. A satisfactory understanding of this phenomenon requires a detailed knowledge of the photochemistry of such molecules. In principle, photochemical reactions can only be anticipated on the basis of good knowledge of the possible photochemical mechanisms. To achieve this goal different types of study have to be undertaken:

(a) Photophysical studies– light absorption and emission (fluorescence, phosphorescence) to determine the nature of involved excited states, as well as their energies. Laser flash photolysis for detection of triplet states of other short-lived transition species that could interact with biomolecules. Singlet oxygen detection (steady state or time resolved near infrared emission).

(b) Photosensitized reactions of biomolecules– photodynamic lipid peroxidation, photomodification of proteins (protein photocrosslinking, drug protein photobinding) drug photosensitized DNA damage (strand breaks, oxidative damage to bases, pyrimidine dimers).

(c) Photochemical studies– photostability, photodegradation (isolation and identification of drug-derived photoproducts by chromatography and spectroscopy) and product-based elucidation of the photochemical mechanisms.

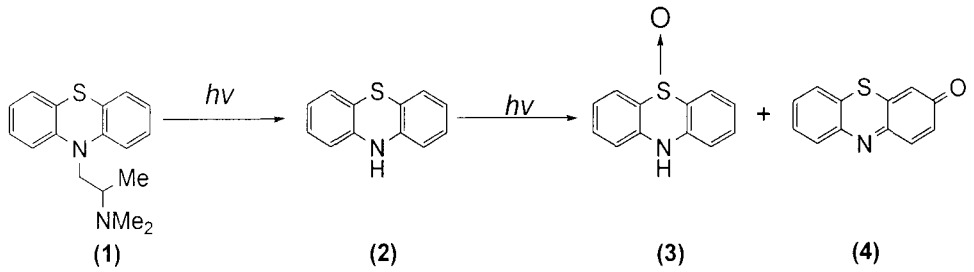
(d) Photooxidation of drug molecules with singlet oxygen.

In the present work we have undertaken two of the above aspects 'c' and 'd' of the drug photochemistry using some representative examples from established drugs and promising drug (biologically active molecules) class.

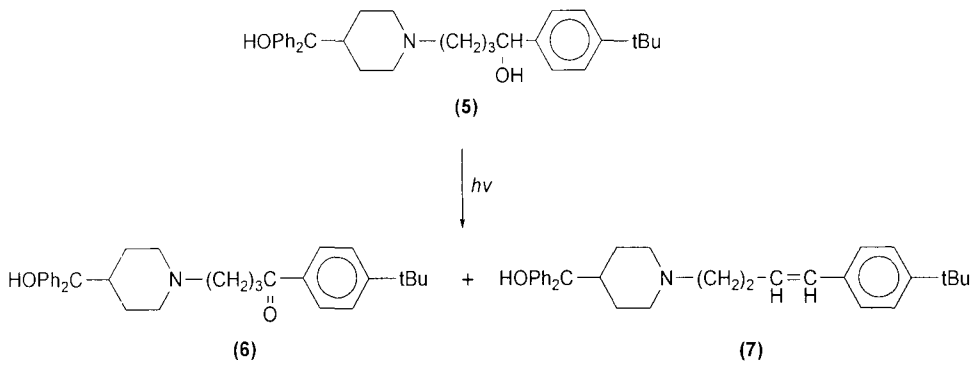
A collection of literature is presented here which illustrate the various *in vitro* photoreactions of the various drugs and related substances.

Anti-histaminic and immunosuppressant drugs:

Among drugs with anti-histaminic action are thiazine derivative promethazine **(1)**, which upon irradiation gets, N-dealkylated to phenothiazine **(2)** which in turn is oxidized to the sulfone **(3)** and to the 3H-phenothiazine-3-one **(4)** (Scheme 1.2).[78] Antihistaminic drug terefenadine **(5)** undergoes oxidation (main process) and dehydration at the benzylic position to give products **(6)** and **(7)**, respectively, upon irradiation in aqueous solution[79] (Scheme 1.3).

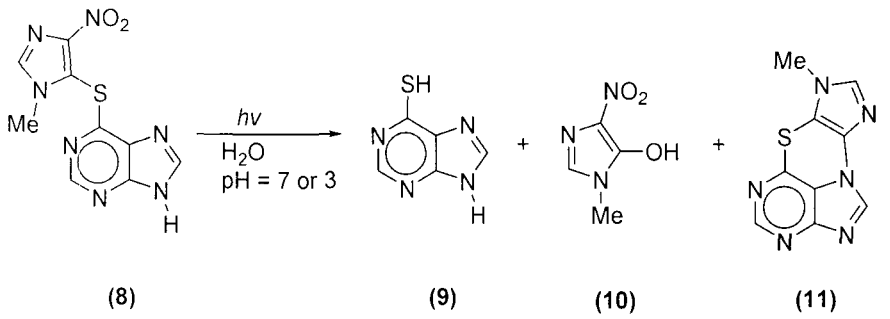


Scheme 1.2



Scheme 1.3

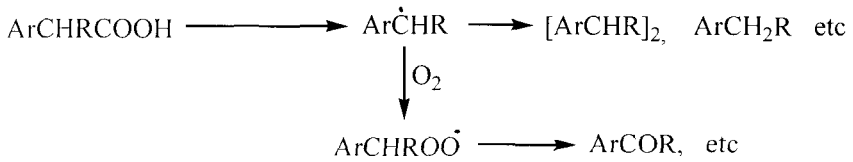
The immunosuppressant drug azathioprene **(8)** undergoes fragmentation of the C-S bond to give 6-mercaptopurine **(9)** and 1-methyl-4-nitro-5-hydroxyimidazole **(10)** as well as cyclization reaction to give **(11)**[80] (Scheme 1.4).



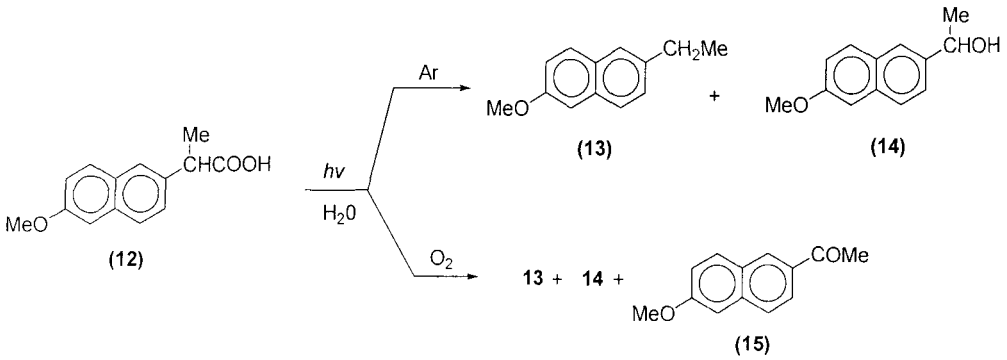
Scheme 1.4

Non steroidal anti-inflammatory drugs:

A variety of 2-aryl propionic acid derivatives are used as anti-inflammatory drugs. Most of these are photoreactive and have some phototoxic action. As a consequence, their photochemistry has been intensively investigated.[81-83] The main process in aqueous solution is decarboxylation to yield a benzyl radical, a general reaction with α-arylcarboxylic acids.[84] Under anaerobic conditions, benzyl radical undergo dimerization or reduction (and in an organic solvents abstracts hydrogen).[85] In presence of oxygen, addition to give hydroperoxy radical and the corresponding alcohol and ketone.[86]

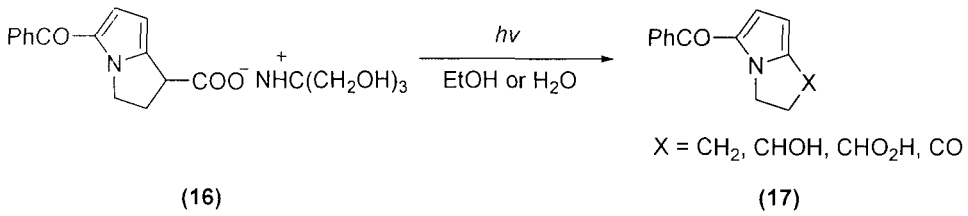


Scheme 1.4



Scheme 1.5

The results from the irradiation of naproxen **(12)** in water are shown in Scheme 1.5, and a related chemical course is followed with several drugs pertaining to this group, such as ibuprofen,[87] butibufen,[88] flurbiprofen,[87] ketoprofen,[89,90] suprofen,[91] benoxaprofen,[88,92] tiaprofenic acid[93] and ketorolac tromethamine **(16)**[94] (Scheme 1.6).

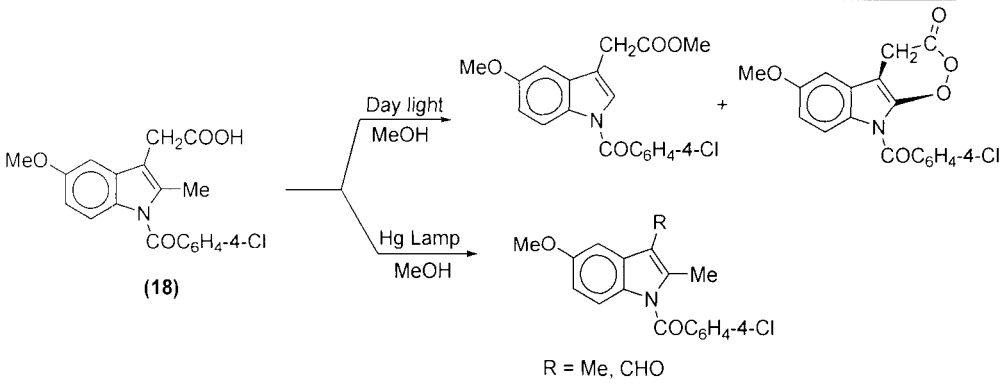


(16) (17)

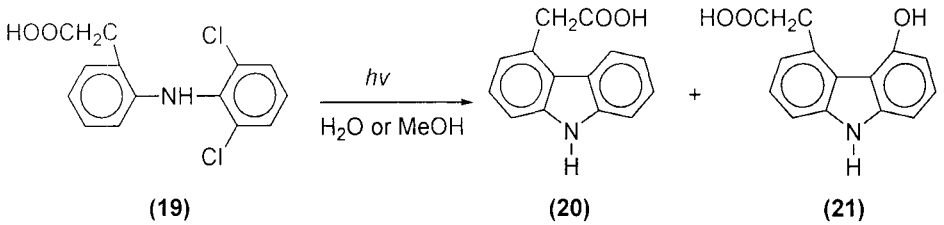
Scheme 1.6

For Indomethacin **(18)** in methanol decarboxylation is the main process,[95,96] when mercury lamps are used, while day light irradiation leads to products conserving the carboxyl group which have been rationalized as arising via the acyl radical (Scheme 1.7)[97]. In case of the related drug diclofenac **(19)**, on the other hand, dechlorination as stated above is the dominant process. Sequential loss of both chlorine atoms is followed by ring closure, reasonably via radical addition, to yield the carbazole-1-acetic acid **(20)** and **(21)** as main products (scheme 1.8).[98] The anti-inflammatory agent meclofenamic acid **(22)** likewise undergoes dechlorination and ring closure to the carbazoles **(23)** and **(24)** (Scheme 1.9).[99]

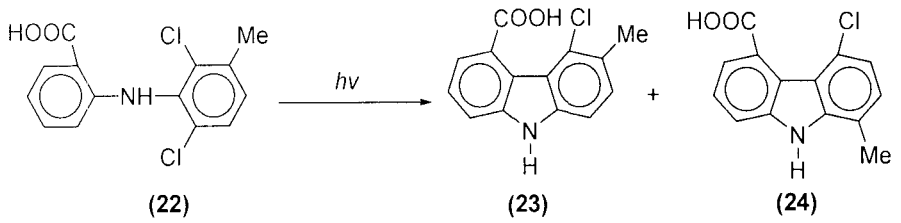
Photoreactivity has been reported also for some anti-inflammatory and analgesic drugs different from aryl acetic acid.[100-102]



Scheme 1.7



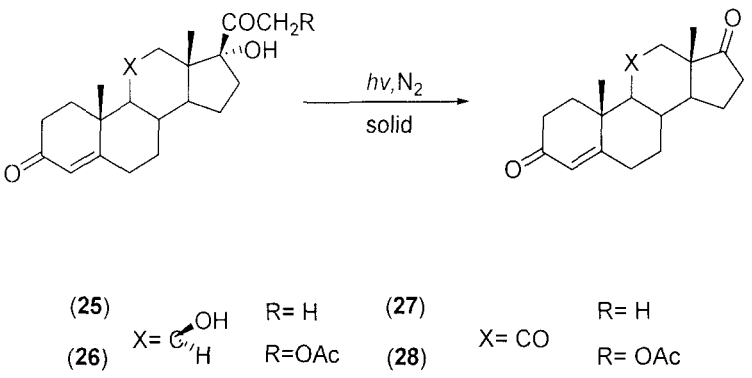
Scheme 1.8



Scheme 1.9

Glucocorticosteroids:

The photoreactivity of glucocorticosteroids have been explored both in solution as well as in solid state and it is well known that they have to be protected from light. Hydrocortisone **(25)**, cortisone **(27)** and their acetates **(26, 28)** undergo photooxidation in the solid state. The main process involves loss of the side chain at C-17 to give androstendione and trione derivatives respectively (Scheme 1.10).[103] Molecular packing has an important role in determining the photostability in the solid. As an example irradiation of crystalline hydrocortisone *tert*- butylacetate leads to the photooxidation of two over five of the polymorphs investigated. This fact has been correlated with the possibility of oxygen to penetrate in the crystal in such structure.[104]



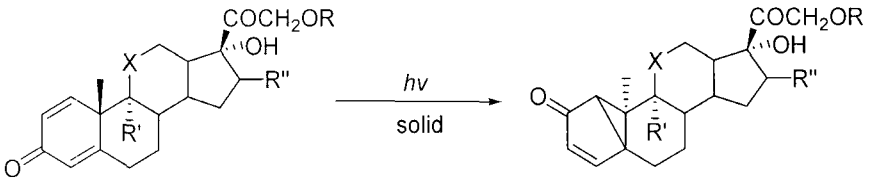
(25) X= OH, H R= H
(26) R=OAc

(27) X= CO R= H
(28) R= OAc

Scheme 1.10

Cross conjugated glucocorticosteroids such as prednisolone **(29)**, prednisone **(30)**, betamethasone **(31)**, triamacinolone **(32)** and others are quite

photoreactive, as one may expect, since the efficient photorearrangement of cyclohexadienones to bicyclo[3.1.0] hexanones is well known.[105] This rearrangement has been observed for dexamethasone, prednisolone, prednisone, betamethasone and some of their acetates (Scheme 1.11).[106,107] The primary photoproducts may undergo further transformation with cleavage of the three membered rings resulting in re-aromatization or cleavage of ring A or in expansion of ring B.



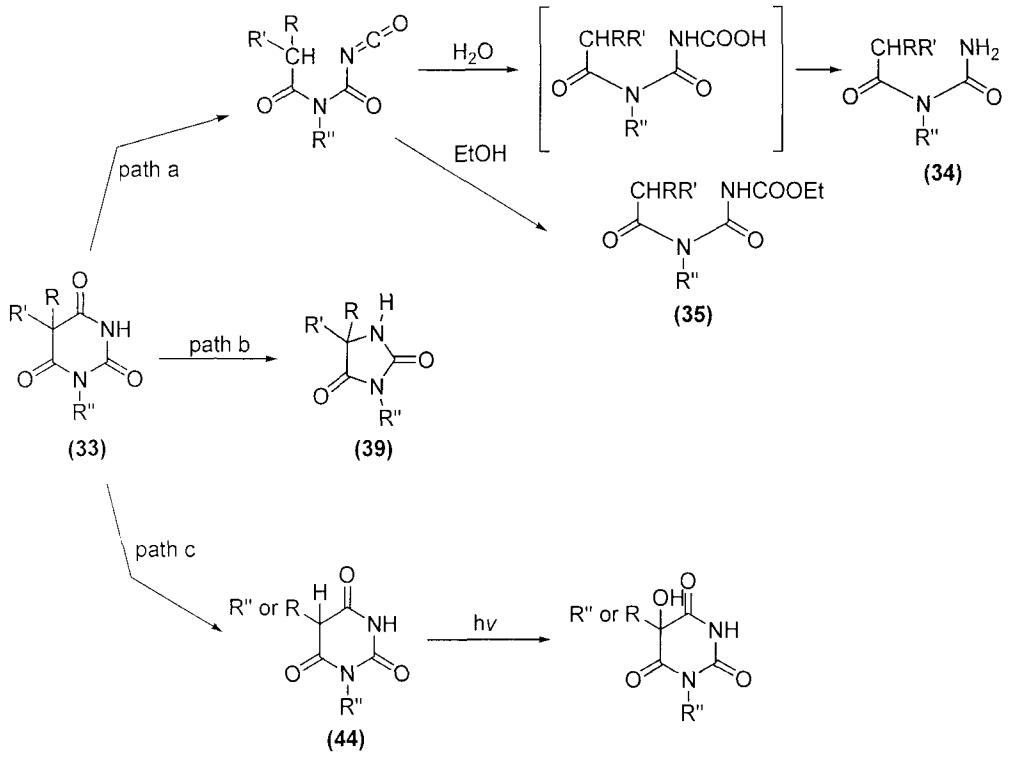
	X	R	R'	R"
(29)	CO	H, Ac	H	H
(30)	C(OH)H	H, Ac	H	H
(31)	C(OH)H	H, Ac	F	Me
(32)	C(OH)H	H	F	Me

Scheme 1.11

Drug acting on central nervous system:

5,5-Alkyl derivatives of barbituric acid **(33)** are used as hypnotic and tranquillizers. These compounds undergo two types of photochemical reactions (Scheme 1.12). In first type of reactions cleavage of the C(4)-C(5) bonds takes place which leads to the formation of an intermediate isocyanate

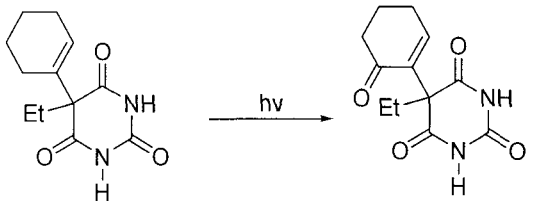
(path a), which in turn adds nucleophilic solvents to give an amide **(34)** in water and urethane **(35)** in ethanol. The positive evidence for the formation of isocyanate has been obtained by irradiation in the solid state.[108] Barton et al.[109] studied similar type of reaction pattern in case of barbital (**36**, R, R'=Et, R''=H) and its methyl derivative (**37**, R, R'=Et, R''=Me). The second one involves cleavage of second C-C bond with the elimination of CO (path b). Barton et al.[110,111] obtained similar reaction pattern with mephobarbital (**38**, R=Et, R'=Me, R''=H) which leads to hydantoin **(39)** as product. Different process occurs for the acidic forms of cyclobarbital **(40)**, which is photooxidized to ketone **(41)** rather than cleaved (Scheme 1.13).



Scheme 1.12

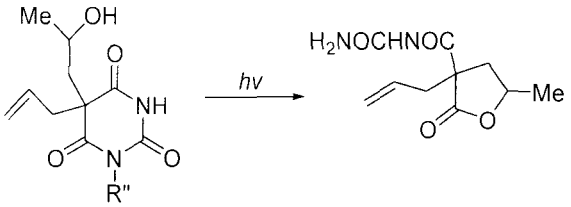
For the tranquillizer proxibarbital **(42)** it was suggested that a nucleophilic group in the side chain intervenes in the process via intermolecular addition

and gives the tetrahydrofuran **(43)** (Scheme 1.14).[112] Monoalkylbarbiturates **(44)** undergo hydroxylation at position 5.[113] 2-Thio analogue of Phenobarbital **(45)** gives **(46)** by selective reduction of the thiocarbonyl function by irradiation in alcohols (Scheme 1.15).[114]



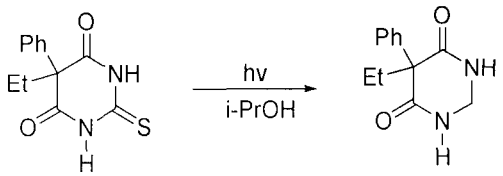
(40) **(41)**

Scheme 1.13



(42) **(43)**

Scheme 1.14

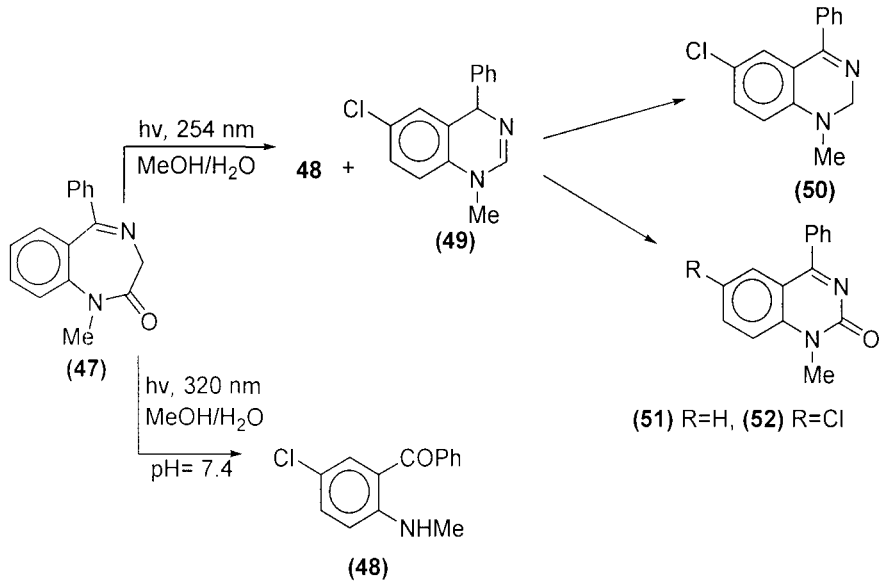


(45) **(46)**

Scheme 1.15

Benzodiazepines:

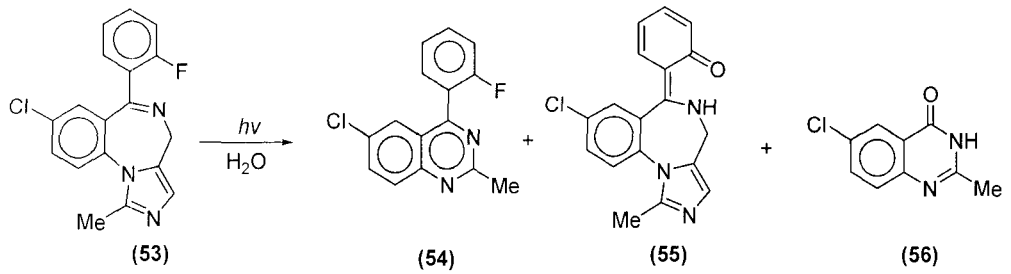
They are generally photolabile, but the path followed in the degradation depends on the structure of each derivative and on the reaction condition. Moore et al.[115] obtained benzophenone **(48)** as main product by the irradiation of diazepam **(47)** at 300 nm in MeOH-H_2O through the cleavage of heterocyclic ring, and dihydroquinazoline **(49)** by irradiation at 254 nm in methanol. This compound then slowly isomerizes to **(50)** as well as to **(51)** and **(52)** through dechlorination and oxidation (Scheme 1.16).



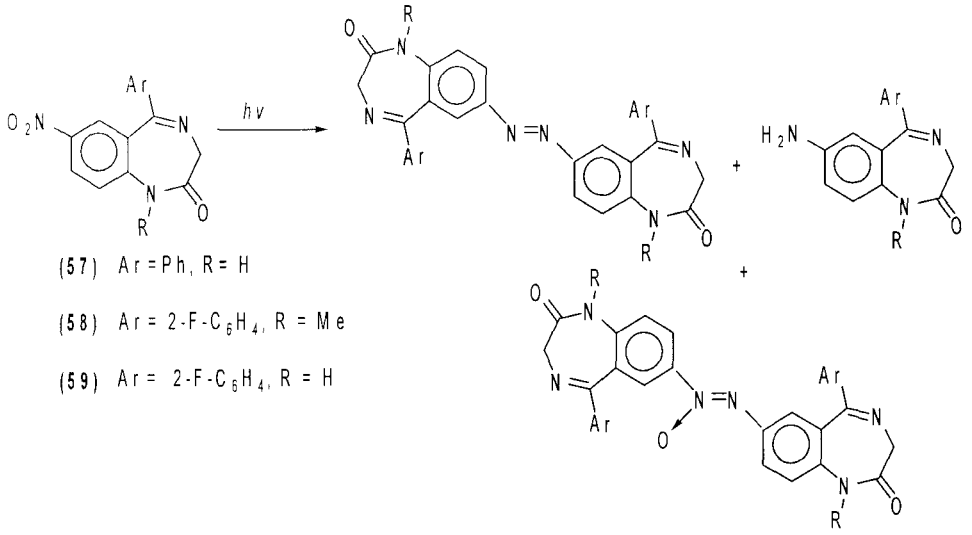
Scheme 1.16

Andersin et al.[116,117] studied intravenous anaesthetic midazolam **(53)** which undergo ring restriction to quinazoline **(54)** as well as oxidation to 5-fluorophenyl moiety to give **(55)** and **(56)** (Scheme 1.17). Roth et al.[118] obtained different reaction pattern in case of nitrazepam **(57)** because of the insertion of nitro subsistent, this abstracts hydrogen and reduced to azoxy, azo and amino function by irradiation in organic solvents under nitrogen. The hypnotic flunitrazepam **(58)** undergoes a multi step reduction finally

leading to the 7-amino derivative under anaerobic conditions.[119,120] While it is N-demethylated to give **(59)** in presence of oxygen[119] (Scheme 1.18).

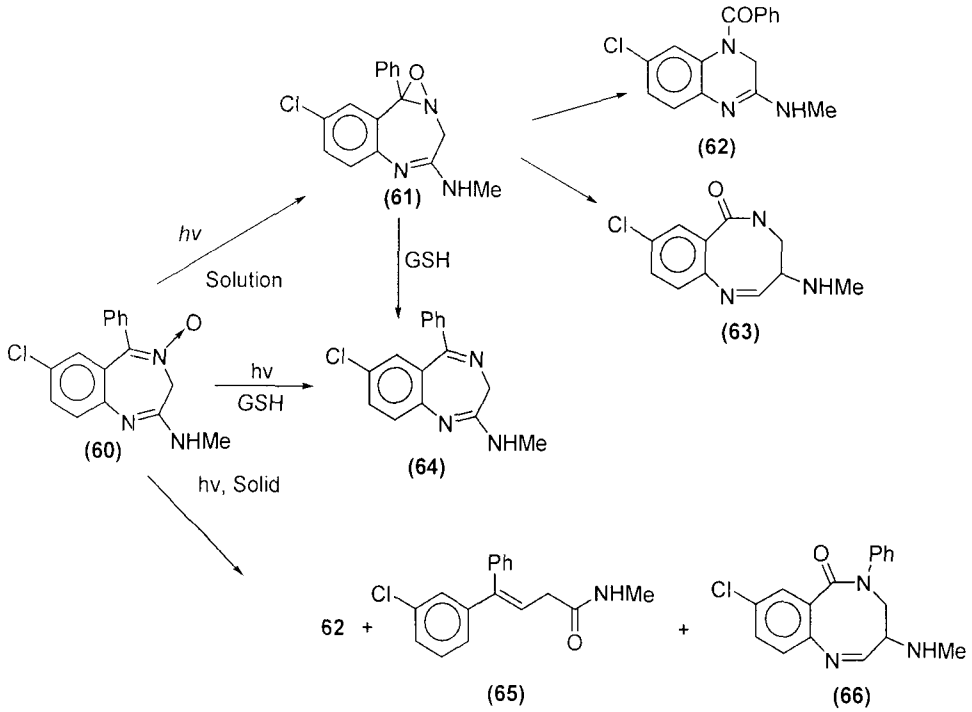


Scheme 1.17



Scheme 1.18

Chlorodiazepoxide **(60)** in which photochemically active moiety N-oxide is present rearranges to oxaziridine **(61)** and further reacts to give compound **(62)** and **(63)** through ring contraction and ring expansion respectively.[121,122] The solid state photochemistry of chlordizepoxide[123] gave quinazoline **(62)** and ring opened product **(65)** and **(66)**. Cornelissen[124] also studied same compound in presence of reducing agent such as glutathione where the main observed process is N-deoxygenation to **(64)** (Scheme 1.19).

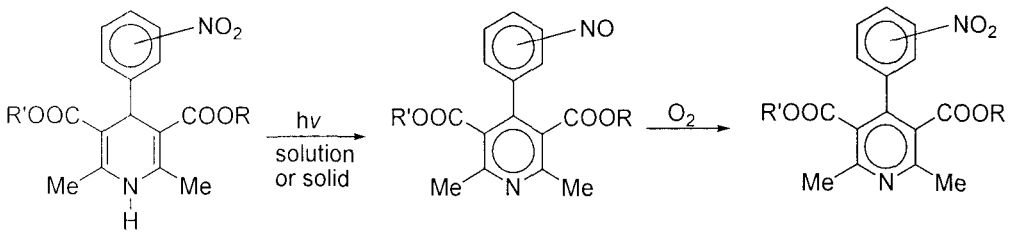


Scheme 1.19

Antihypertensives:

4-nitrophenyldihydropyridines are widely used as vasodilators and due to presence of hydrogen abstracting nitro group and easily abstracting benzylic hydrogen these drugs are considered to be quite photoreactive. Reduction of nitro to nitroso group of heterocyclic ring and re-oxidation of nitroso group in the presence of oxygen are mainly observed reaction paths (Scheme 1.20). Nifedipine **(67)**, the 4-nitrophenyldihydropyridine derivative, shows similar type of reaction pattern.[125-127] Other nitrophenyldihydropyridine derivative

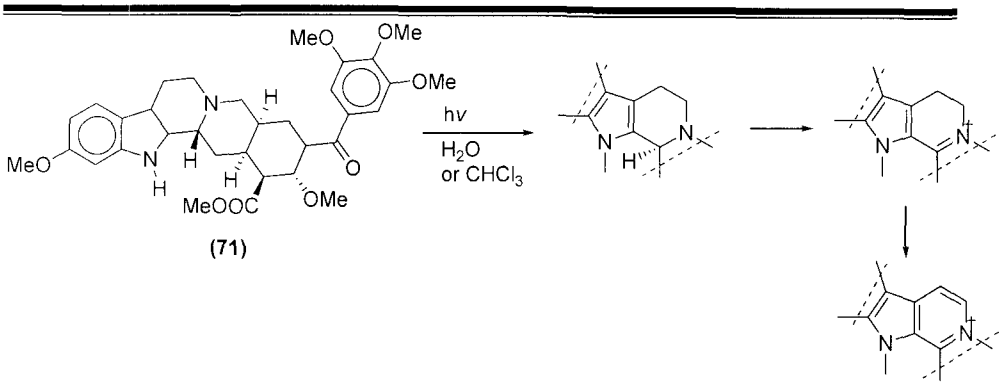
such as nicardipine **(68)**,[128] furnidipine **(69)**[129] and nimodipine **(70)**[130] also photoreact in the similar manner.



(67) R,R' = Me, 2-NO_2
(68) R= Me, R' =[(N-methyl-N-benzyl)-2-aminoethyl]
(69) R,R' =Et, 2-tetrahydrofurylmethyl
(70) R, R' =*iso*-propyl, 2-methoxyethyltetrahydrofurylmethyl

Scheme 1.20

Diltiazem is stable in solid state[131] but on irradiation of aqueous solution at different pH it gets deacetylated. Karlicek et al.,[132] on irradiation of aqueous solution of ergotamine under nitrogen atmosphere, obtained product due to hydration of double bond at 9,10 position. Reserpine **(71)** photoreacts both in aqueous solution and in chloroform and the process occurring are epimerization at C-3 and stepwise dehydration of the tetrahydro carboline skeleton[133,134] (Scheme 1.21).

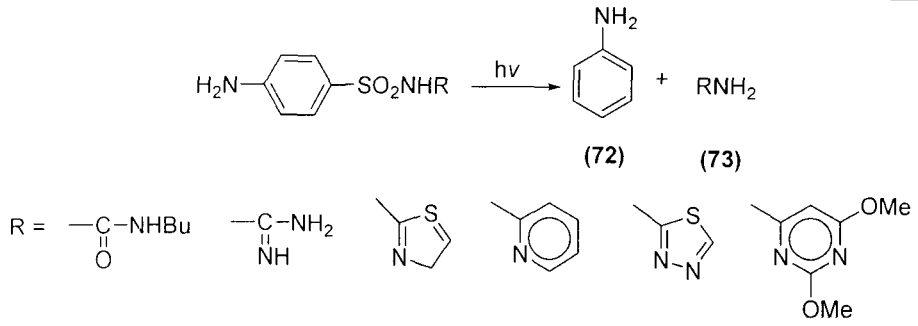


(71)

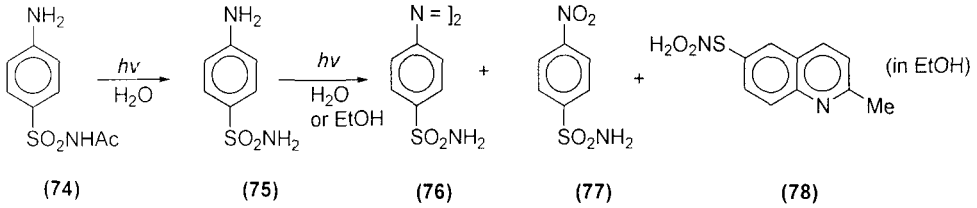
Scheme 1.21

Antibacterials:

Golpashin et al.[135] studied several N-substituted sulfanilamide derivative and reported that yields of products changes with the structure. The main process takes place is the cleavage of C-S and S-N bonds with the elimination of SO_2 and formation of aniline **(72)** and appropriate amine **(73)** (Scheme 1.22). Chiang et al.[136] observed that methylation of amino group take place on irradiation of sulfadimetoxine in methanol. Irradiation of sulfacetamide **(74)** in water yielded deacetylated product sulfanilamide **(75)**, which undergoes oxidation of the amino group to give the azo **(76)** and the nitro **(77)** derivative,[137] when irradiated in water. In ethanol[138] the formation of 2-methylquinoline-6-sulfonamide **(78)** accompanies the above process (Scheme 1.23).

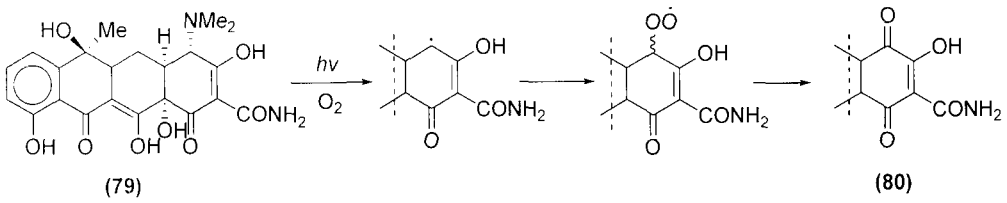


Scheme 1.22



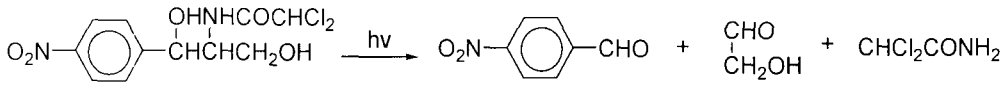
Scheme 1.23

Darvis et al.[139] and Moore et al.[140] carried out irradiation of aqueous solution of tetracycline **(79)** under aerobic condition where homolytic deamination with the addition of oxygen, resulted in the formation of quinine **(80)** (Scheme 1.24). Chlorotetracycline on irradiation in aqueous buffer solution at pH 7.4 undergo homolytic dechloroniation.[141]



Scheme 1.24

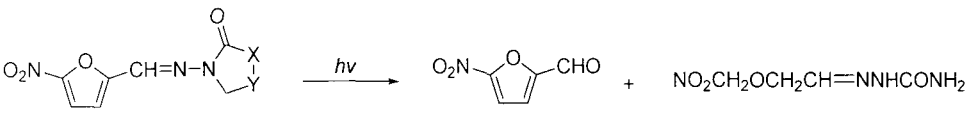
Aqueous solution of chloramphenicol **(81)** gives 4-nitrobenzaldehyde **(82)**, glycolic aldehyde **(83)** and dichloroacetamide **(84)** by the homolytic cleavage of C-C bond. The resulting product 4-nitrobenzaldehyde undergoes secondary photoreaction and leads to amino and nitro benzoic acid as well as aminobenzaldehyde oxime[142-144] (Scheme 1.25).



(81) **(82)** **(83)** **(84)**

Scheme 1.25

The urinary anti-bacterial nitrofurantoin **(85)** is cleaved to nitrofuran carboxaldehyde upon UV irradiation. Furazolidone **(86)** is cleaved and hydrolyzed to nitrofuran carboxaldehyde (Scheme 1.26). [145]

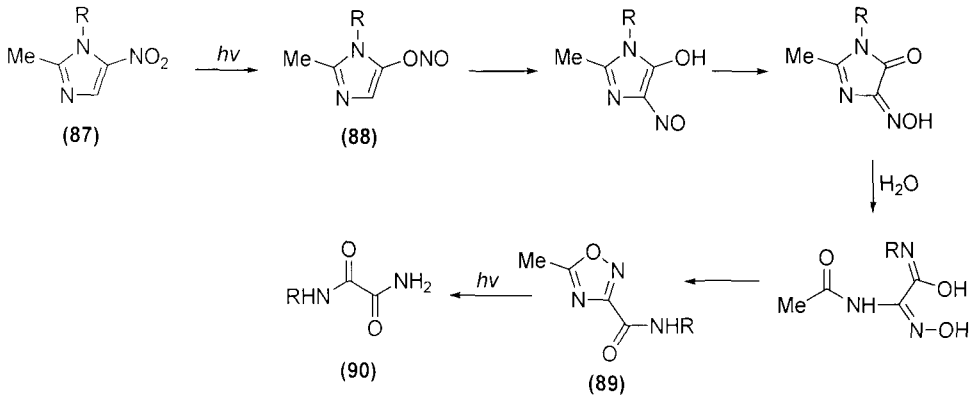


(85), X = NH, Y = CO
(86), X = O, Y = CH_2

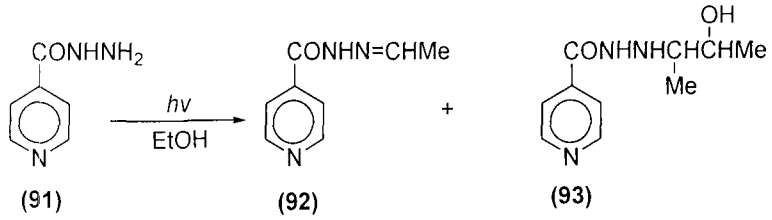
Scheme 1.26

Among five membered heterocycles metronidazole **(87)** and related antibacterials show typical nitro group photoreactions.[146-148] This is initiated by typical nitro-nitrite rearrangement to give **(88)** followed by shift of NO group to vicinal position. Hydrolytic ring opening-ring closure from oxime

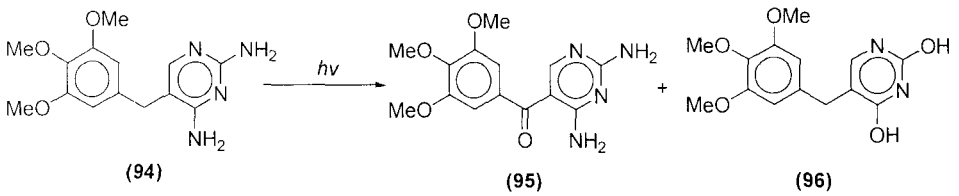
leads to 1,2,4-oxadiol-3-carboxamide **(89)** which finally hydrolyzed to oxaldiamides **(90)** (Scheme 1.27). A different photochemistry of metronidazole was observed in presence of citrate. [149] Irradiation of six membered heterocyclic drug isoniazid **(91)** in ethanol caused oxidation of ethanol to acetaldehyde which is trapped by substrate to yield hydrazone **(92)** which again reacts with a molecule of ethanol to give **(93)**[150] (Scheme 1.28). Trimethoprim[151] **(94)** undergoes oxidation at benzylic position and hydrolysis of amino group in pyrimidine ring to yield **(95)** and **(96)** (Scheme 1.29).



Scheme 1.27



Scheme 1.28



(94) (95) (96)

Scheme 1.29

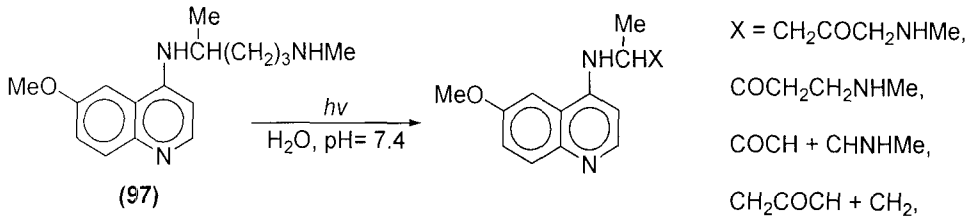
Fluoroquinolones:

Quinolone antibiotics bearing fluorine substituent, such as norfloxacin **(96a)** are commonly called fluoroquinolones. The photobehaviour of fluoroquinolone antibiotics has recently been the object of increasing interest due to the finding of their photosensitizing properties.[152] The main result obtained for a series of structurally related, representative fluoroquinolone drugs is reviewed.[153] Both activation of oxygen and various degradation pathways have been identified and the effects of medium and structure have been rationalized. The results can help in the understanding of the photochemistry occurring in biological environments and in the assessing of the correlation between structural characteristics and biological photodamage.

Antiprotozoal drugs:

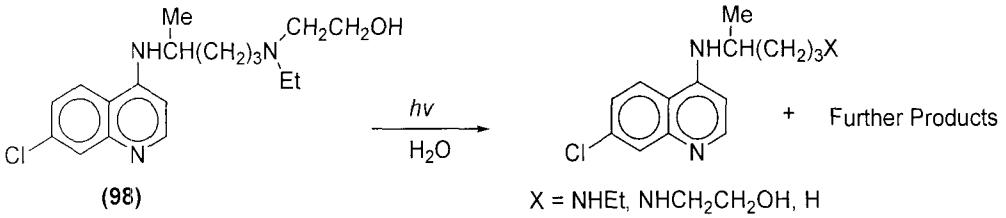
Photochemistry of antiprotozoal drug quinine occurring in citric acid solution has been extensively studied[154] and this drug is well known to be photolabile.[155] Photochemical oxidative degradation of alkyl amino chain is the main reaction path observed in the majority of this class drug. 6-Alkylamino derivative primaquine **(97)**,[156,157] 4-alkylamino derivative

amodiaquine,[158] hydroxychloroquine **(98)**[159] and chloroquine **(99)**[160-162] undergo degradation of alkylamino side chain (Scheme 1.30, 1.31 and 1.32). Tonnesen and Grislingaas[163] found that mefloquine **(100)**, on irradiation in methanol produces **(101)** and **(102)** (Scheme 1.33).



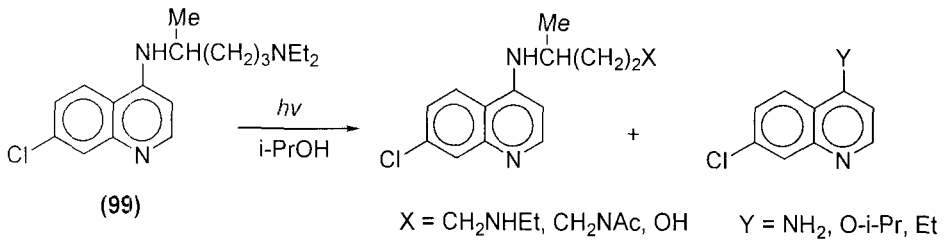
(97)

Scheme 1.30

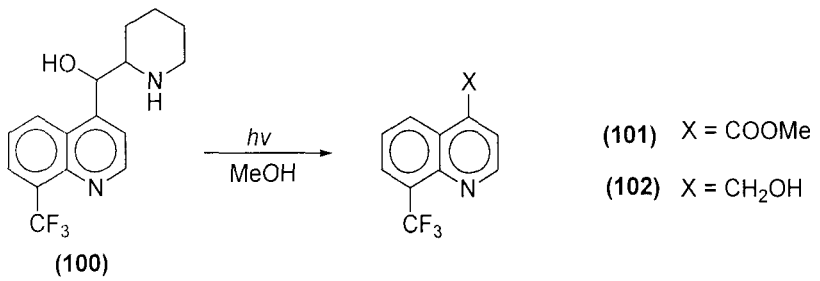


(98)

Scheme 1.31



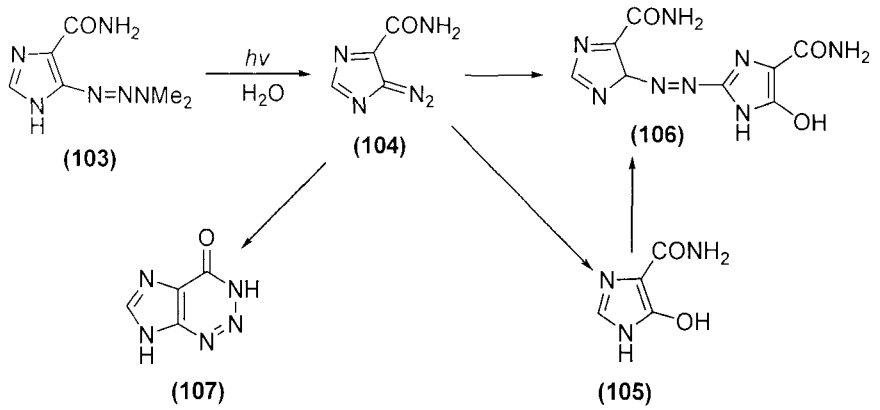
Scheme 1.32



Scheme 1.33

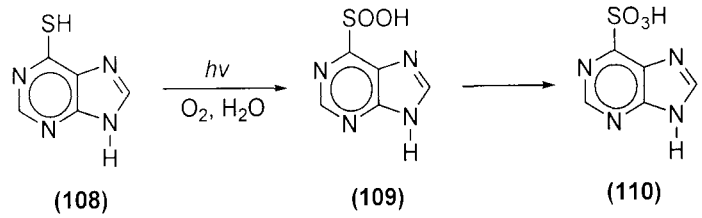
Antineoplastic drugs:

Horton et al.[164] observed that irradiation of dacarbazine **(103)** in solution causes dimethylamine elimination to yield diazo derivative **(104)**, which is further hydrolyzed to give hydroxyimidazole **(105)**. This hydroxyimidazole in turn couple with diazo derivative **(104)** to give azo derivative **(106)**. Alternatively **(104)** cyclize to give 2-azahypoxanthine **(107)** (Scheme 1.34).



Scheme 1.34

An investigation of photochemistry of anti-tumor antibiotics, hedamycin and kidamycin has been reported.[165] 6-Mercaptopurine **(108)** is oxidized when irradiated in oxygen equilibrated aqueous solution by near U.V light giving sulfinate **(109)** as the primary product, which is finally oxidized to sulfonate **(110)** (Scheme 1.35).[166] Several other anti-neoplastic drugs as alkaloid vinblastine sulphate, carmustine, tauromustine and mitonaftide were found to be photochemically unstable.



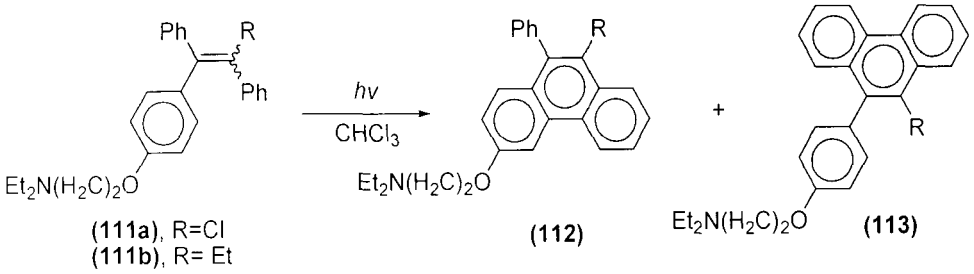
Scheme 1.35

The neuroleptic drug levomepromazine is photolabile under UV-A and UV-B light in aerobic conditions. Irradiation of a methanol solution of this drug produces one photoproduct, resulting from the oxidation of levomepromazine. It is demonstrated that photodegradation occurs via type II mechanism involving irreversible trapping of self-photogenerated singlet molecular oxygen.[167]

The photoreactivity of phototoxic anti-cancer drug flutamide has also been studied in homogenous media, cyclodextrin cavity and liposomes.[168,169]

Gonadotropic steroid and synthetic estrogens:

Synthetic estrogens are generally the stilbene derivatives. They undergo fast E-Z photoisomerization, and *trans* fused dihydrophenanthrene is formed by the conrotatory electrocyclic ring closure of the Z-isomer, which in turn gets aromatized in presence of oxidants such as atmospheric oxygen. Thus clomiphene **(111a)**, when irradiated in chloroform solution gives phenanthrenes **(112)** and **(113)**[170] (Scheme 1.36). Cyclization is also observed with temoxifen **(111b)**.[171] Dihydroxy substituted stilbenes such as dienoestrol **(114)** and stilboestrol **(115)** are like wise cyclized[172-174] (Scheme 1.37 and 1.38).

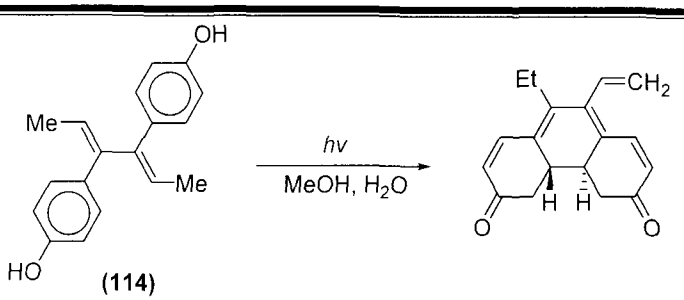


(111a), R=Cl
(111b), R= Et

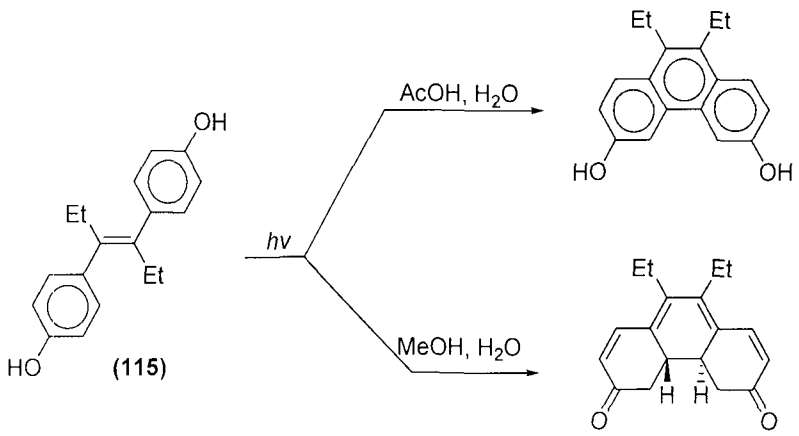
(112)

(113)

Scheme 1.36

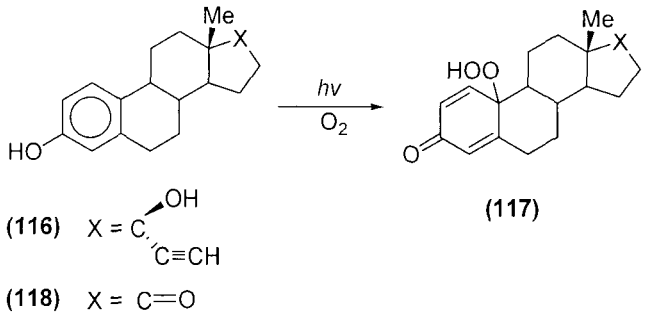


Scheme 1.37



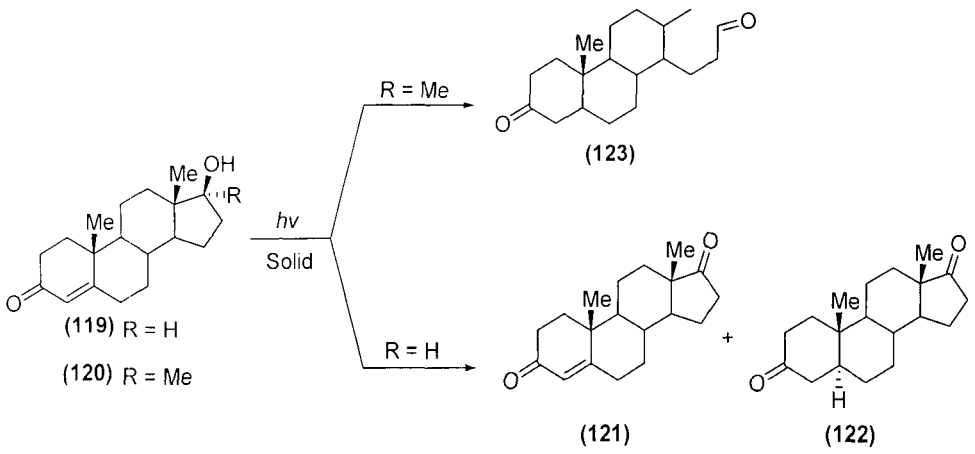
Scheme 1.38

The phenolic ring present in the estrogens makes them quite labile to photooxidation. The reaction can be conveniently carried out by photosensitization, under conditions where singlet oxygen is produced avoiding direct irradiation of the substrate. Sedee and co-worker[175] observed that photosensitized reaction of estradiol **(116)** involve singlet oxygen addition to electron rich phenolic ring to yield ketohydroperoxide **(117)**. Similar reaction pattern was obtained in case of estrone **(118)**[176] (Scheme 1.39).

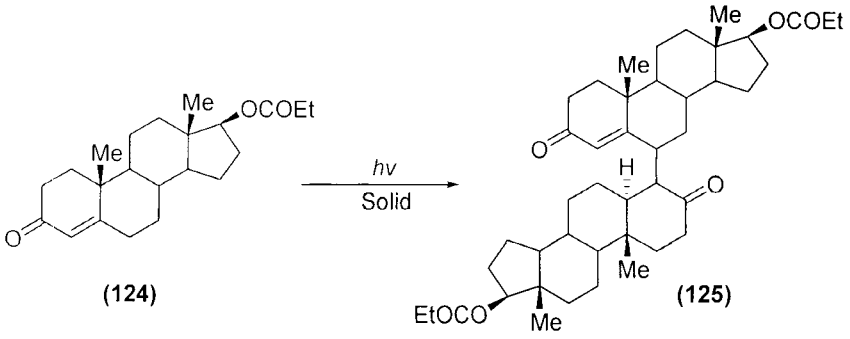


Scheme 1.39

When photolysis is carried out in solid state different reaction paths are observed. Reisch et al.[177,178] have reported that testosterone **(119)** yielded androstenedione **(121)** and androstanedione **(122)**, while 17- methyltestosterone **(120)** gives seco-derivative **(123)** (Scheme 1.40). Levonorgestrol and ethisterone are photodimerized in solid state under nitrogen atmosphere.[179,180] Testosterone propionate **(124)** gives dimer **(125)** when irradiated in solid state (scheme 1.41).[181]



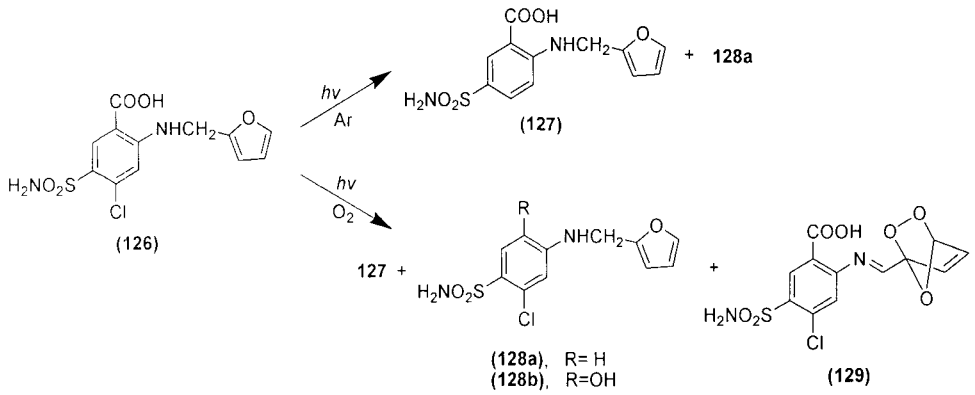
Scheme 1.40



Scheme 1.41

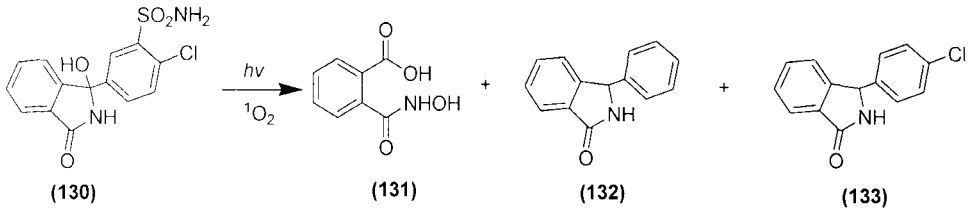
Anti-diuretic Drugs

The phototoxic diuretic drug furosemide **(126)** is photolabile under aerobic and anaerobic conditions. Irradiation of methanolic solution of furosemide under oxygen atmosphere gave photoproducts **127**, **128a**, **128b** and **129**, while under argon atmosphere the photoproduct **127** and **128a** were isolated[182] (Scheme 1.42). A peroxidic unstable product was also detected during the photolysis under oxygen atmosphere. Furthermore, a photocycloaddition of singlet oxygen to furan group of furosemide was also detected.



Scheme 1.42

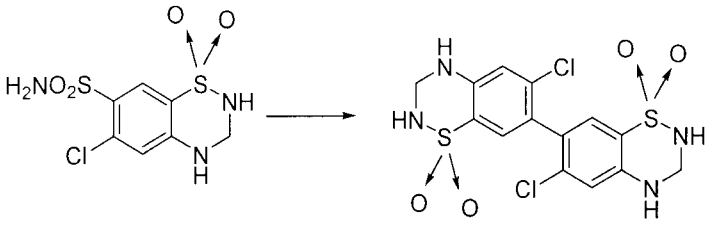
The phototoxic diuretic drug chlorthalidone **(130)** is photolabile under aerobic conditions and UVB light.[182,183] Irradiation of this drug under oxygen atmosphere produces photoproducts **131**, **132**, **133** and singlet oxygen (Scheme 1.43).



(130) (131) (132) (133)

Scheme 1.43

The phototoxic antidiabetes drug glipizide, a benzosulfonamide derivative, was also photolabile under aerobic conditions and UVB light.[184] Hydrochlorothiazide **(134)** dimerized[185] upon irradiation with a medium pressure Hg arc lamp through a glass filter in deaerated aqueous or alcohol solution to give **(135)** (Scheme 1.44).



(134) (135)

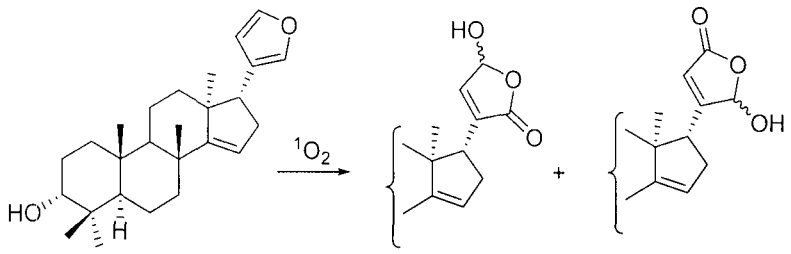
Scheme 1.44

Photooxidation of troglitazone, an antidiabetic drug, gave the quinone and quinone epoxide as the major products.[186] Photooxidation of the ophthalmic drugs pindolol

and timolol in water was reported to involve singlet oxygen.[187] Photooxidative degradation of papaverine [188] and clomipramine[189] has also been reported.

Plant derived natural products

The neem triterpenoid nimbin **(136)** on photo-oxidation produced two isomeric products (**137**, **138**) containing a hydroxybutenolide moiety, formed by the reaction of singlet oxygen with the furan ring.[190] (Scheme 1.45).

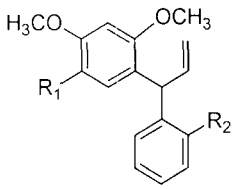


(136) **(137)** **(138)**

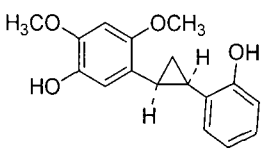
Scheme 1.45

Latifolin **(139)**, the major constituent of D. latifolia on photolysis gave *trans*-1-(2,4-dimethoxy-3-hydroxyphenyl)-2-(2-hydroxyphenyl) cyclopropane **(140)** as the sole photo di-Π-methane rearrangement product. In contrast, its analogues, 3-(2,4,5-trimethoxyphenyl)-3-phenyl-prop-1-ene **(141)** and 3-(2,4-dimethoxyphenyl)-3-phenyl-prop-1-ene **(142)**, gave a 1:1 mixture of *cis*- and *trans*-cyclopropanes. Dye-sensitized photooxidation of latifolin and dihydrolatifolin **(143)** gave novel xanthan derivatives **(144)** and **(145)** involving a crucial step of photooxidative demethylation followed by cyclization. Similar reaction of the closely related propenyl compound **146** gave, interestingly the benzofuran **(147)**. The propane **(148)**, lacking free hydroxyl or double bond, gave only the quinone **(149)**, indicating that

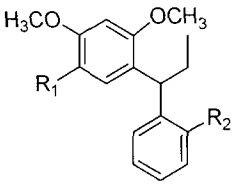
quinones are intermediates in the above oxidations. The cinnamyl alcohol (**150**), having similar feature, undergoes oxidation to the corresponding aldehyde (**151**) and the benzophenone (**152**) but not to a quinone[191] (Scheme 1.46).



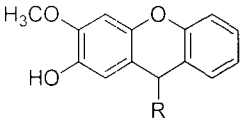
(139), $R_1=R_2=OH$
(141), $R_1=OCH_3$, $R_2=H$
(142), $R_1=R_2=H$



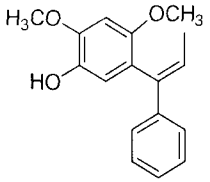
(140)



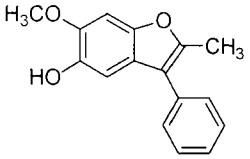
(143), $R_1=R_2=OH$
(148), $R_1=OH$, $R_2=H$



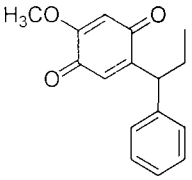
(144), R= $CH=CH_2$
(145), R= CH_2CH_3



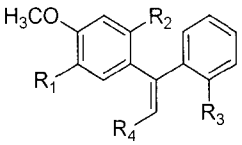
(146)



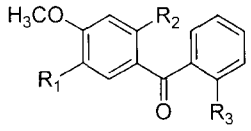
(147)



(149)



(150), $R_1=R_3=H$, $R_2=OH$, $R_4=CH_2OH$
(151), $R_1=R_3=H$, $R_2=OH$, $R_4=CHO$

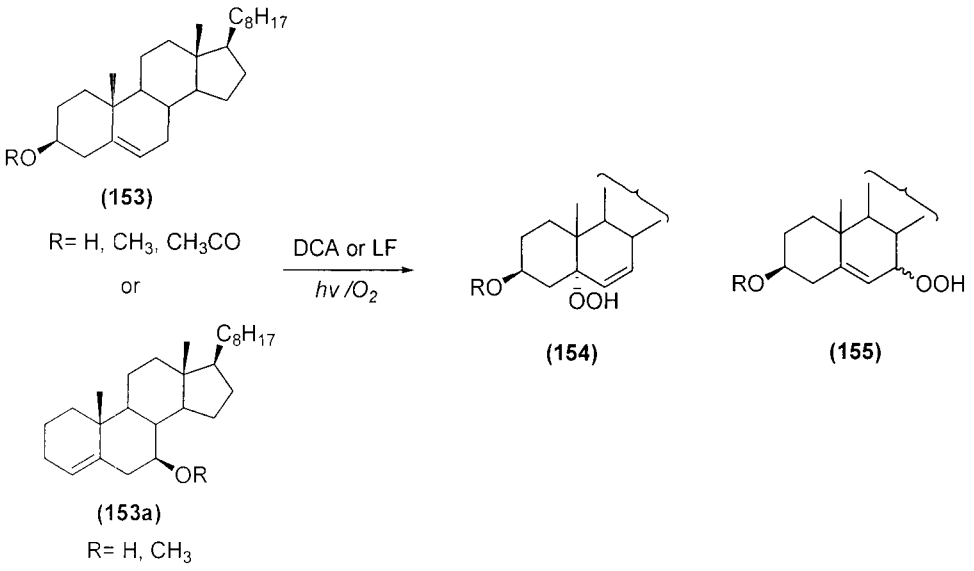


(152), $R_1=R_3=H$, $R_2=OH$,

Scheme 1.46

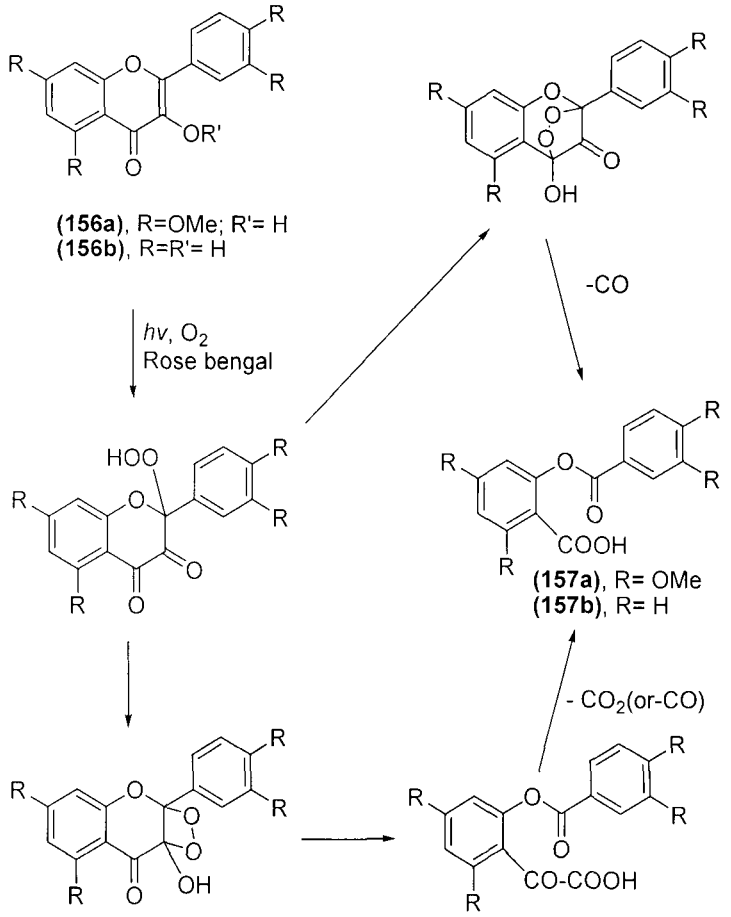
3-Substituted cholesterols (**153**) and 7-substituted pseudocholesterols (**153a**) undergo a facile photooxygenation sensitized by 9, 10-dicyanoanthracene (DCA) and lumiflavin (LF) to give similar, oppositely-positioned enol derivatives (**154**, **155**) (Scheme 1.47). Both steroids showed the same

reaction pattern associated with the endocyclic 5- and 4-olefin units, respectively. The reaction was proposed to proceed via the ene reaction of singlet oxygen and subsequent rearrangement of the initially formed 5α-hydroperoxides.[192]



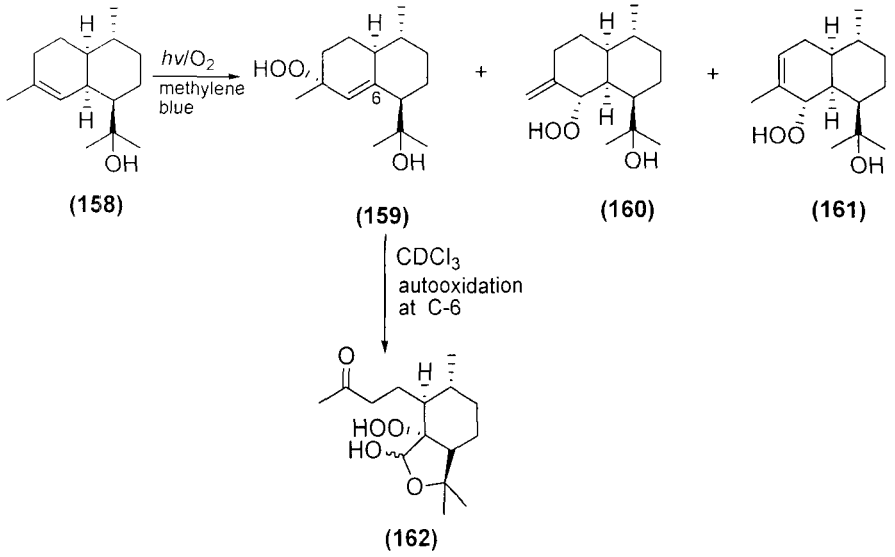
Scheme 1.47

Photosensitized oxygenation of 3-hydroxyflavones **156a** and **156b** in the presence of rose bengal, gave corresponding depsides **157a** and **157b**, carbon monoxide, or carbon dioxide[193] (Scheme 1.48).



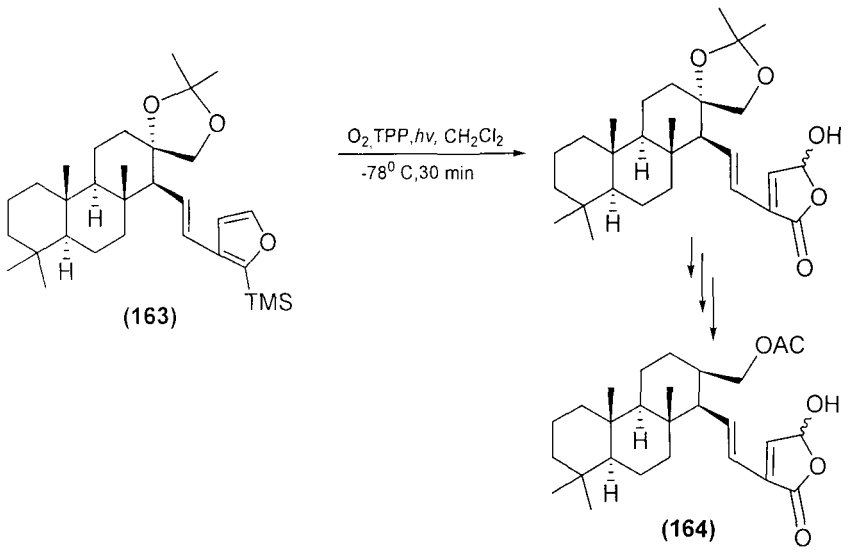
Scheme 1.48

Photooxidation of 4-amorphen-11-ol (**158**), one of the major sesquiterpene natural products from the medicinal plant *Fabiana imbricata*, results in three allylic hydroperoxides[194] (**159**, **160** and **161**), which are expected from the "ene-type" reaction of molecular oxygen with the tri-substituted double bond in **158**. The tertiary allylic hydroperoxide **159** undergoes carbon-carbon bond cleavage and a second autoxidation reaction to yield the more highly oxygenated *seco*-amorphane **162** under mild conditions (Scheme 1.49).



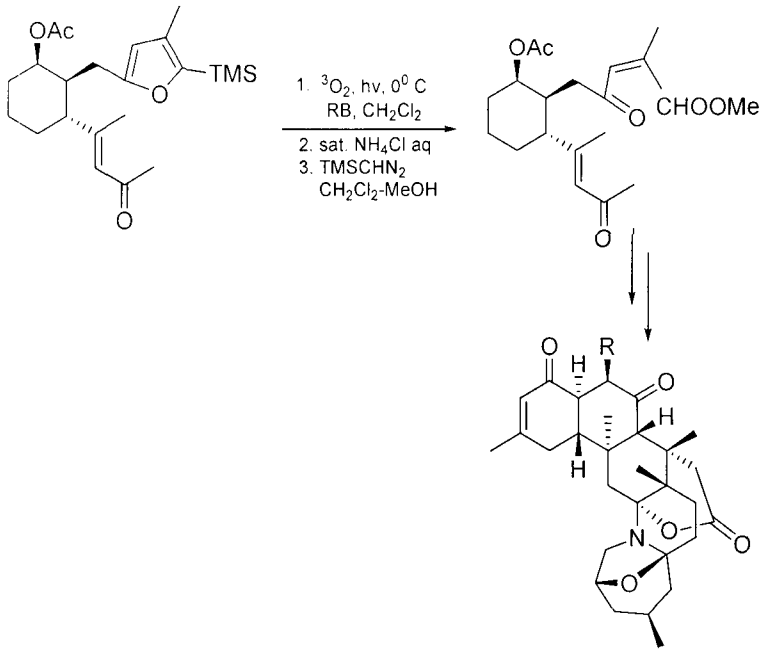
Scheme 1.49

The key step in the synthesis of spongianolide A **(164)**, an antitumoral natural sesquiterpenoid, involved the photooxygenation of trimethylsilylfuran[195] **(163)** as shown in Scheme 1.50.



Scheme 1.50

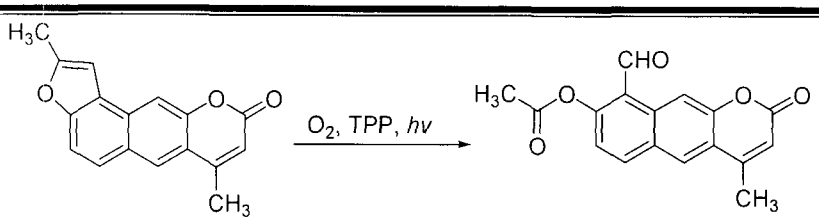
A stereoselective synthesis of zoanthamine **(165)**, potent anti-inflammatory alkaloid, was accomplished by a strategy involving photosensitized oxidation of furan derivative in the key step[196] (Scheme 1.51).



(165)

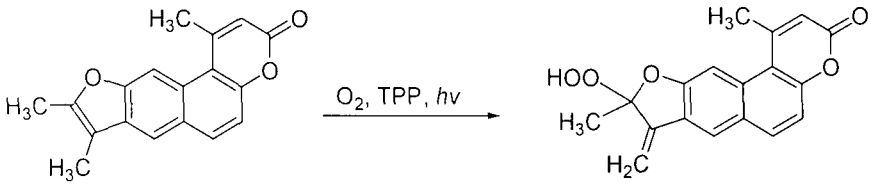
Scheme 1.51

Sometimes dioxetanes are the primarily detected as photooxygenation adducts in the reaction of benzofurans,[197] naphthofurans, naphthodifurans,[198] furonaphthopyrones as **(166)**, which finally leads to characteristic cleavage products (Scheme 1.52). An anomalous behaviour has been observed in the photooxygenation of furanopyrone **(167)**. Indeed differently from **166** which give the corresponding dicarbonyl compound, **167** lead to stable hydroperoxide **(168)** by an ene-type reaction[199] (Scheme 1.53).



(166)

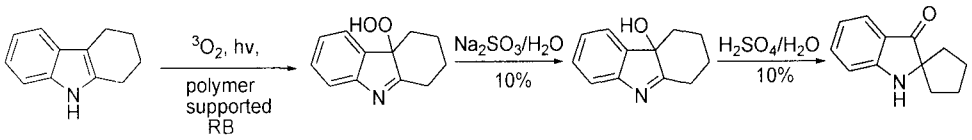
Scheme 1.52



(167) (168)

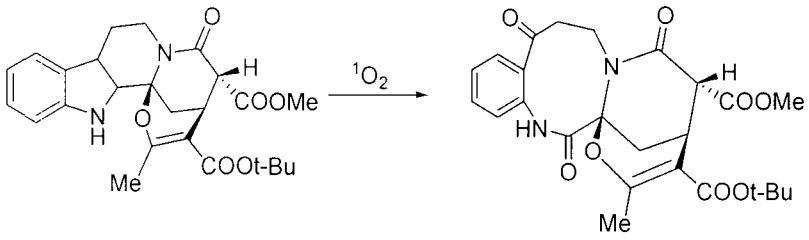
Scheme 1.53

The most significant application of the photooxygenation of indole derivatives is in the synthesis of alkaloids.[200] The photooxygenation of 1,2,3,4-tetrahydrocarbazole is the starting step in the synthesis of spiro derivatives, which would be used as a starting material for the synthesis of spiro analogues of the ergot alkaloid (Scheme 1.54).



Scheme 1.54

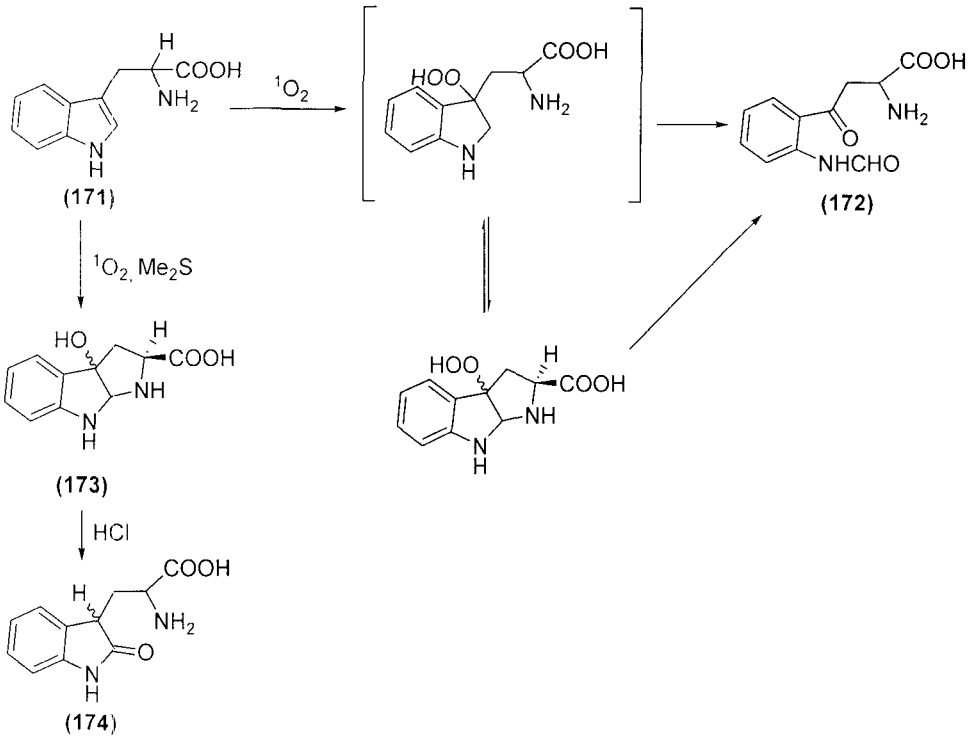
The photooxidative double bond cleavage has been usefully adopted for the construction of large heterocyclic rings containing a carbonyl group. Utilizing this type of reaction the quinine alkaloid camptothecin has been synthesized by quinoline, obtained via photooxygenation of indole **(169)**, followed by the basic treatment of the resulting keto-amide **(170)**[201] (Scheme 1.55).



(169) **(170)**

Scheme 1.55

Tryptophan **(171)** on reaction with oxygen molecule in the presence of riboflavin or methylene blue as a photosensitizer yields N-formylkynurenine **(172)** and its derivatives.[202,203] The hydroxide **(173)** has also been found by reduction of the crude oxygenation mixture, it gives oxytryptophan **(174)** under acidic conditions[204](Scheme 1.56).



Scheme 1.56

The variety in molecular structure of the phototoxic drugs is immense, and almost all classes of drug compounds contain members with adverse photobiological effects. A knowledge of the part of the molecular structure which is responsible for the phototoxicity of a particular drug can provide the opportunity to alter a phototoxicon in such a way that the adverse photobiological effects diminish while the desired ones remain conserved. This aim can be reached more efficiently by combining photoreactivity data from both *in vitro* and *in vivo* investigations. The in vivo system is too complicated and without continuous help from in vitro research, the investigation of this cannot provide much insight. Therefore, we have carried out *in vitro* photochemical studies on certain synthetic drugs and biologically active natural products, as described in the following chapters.

References

1. http:// www.Pol-us.net/Asp_Home/asp_br02.html
2. H. D. Roth, *Angew. Chem.*, 1989, 101, 1220; *Angew. Chem. Int. Ed.*, 1989, **28**, 1193.
3. W. H. Horspool, P. S. Song, CRC Handbook of Organic Photochemistry and Photobiology, CRC press, Boca Raton, 1995.
4. J. D. Coyle, R. R. Hill, D. R. Roberts, Light Chemical Changes and Life: a Source Book in Photochemistry, The Open University press, Milton Keynes, 1992.
5. J. Kopecky, Organic Photochemistry: a Visual Approach, VCH publ., New York, 1992.
6. W. H. Horspool, D. Armesto, Organic Photochemistry: a Comprehensive Treatment, Ellis Harwood, New York, 1992.
7. N. J. Turro, Modern Molecular Photochemistry, Benjamin Cummings, Menlo park, 1978.
8. R. K. Kole, H. Banerjee, A. Bhattacharyya, A. Chowdhury, N. A. Chaudhury, *J. Ind. Chem. Soc.*, 1999, **76**, 595.
9. C. G. Daughton, T. A. Ternes, *Environ. Health Pers.*, 1999, **107**, 907.
10. N. Pandit, 'Chemical Stability of Pharmaceuticals' K. A. Connors, G. L. Amidon, L. Kennon Ed., John Willey and sons, New York, 1979, p 201.

11. H. De Vries, G. M. J. Beijersbergen van Henegouwen, F. A. Huf, *Int. J. Pharm.*, 1984, **20**, 265.

12. J. G. Bartlett, *Med. Clin. North Am.*, 1982, **66**, 91.

13. M. R. Dobrinska, In 'Chemical Stability of Pharmaceuticals' K. A. Connors, G. L. Amidon, L. Kennon Ed., John Willey and sons, New York, 1979, p 208.

14. R. A. Larson, *Phytochemistry*, 1988, **27**, 969.

15. J. P. Knox, A. D. Dodge, *Phytochemistry*, 1985, **24**, 889.

16. H. Kautsky, H. De Bruijn, *Naturwiss.*, 1931, **19** 1043.

17. J. Jasiczak, M. A. Smoczkiewica, *Tetrahedron Lett.*, 1985, **26**, 5221.

18. A. G. J. Sedee, G. B. Van Henegouwen, K. J. Lusthof, G. Lodder, *Biochem. Biophys. Res. Commun.*, 1984, **125**, 675.

19. A. Sedee, G. B. Van Henegouwen, Arch. Pharm., 1985, **318**, 111.

20. C. S. Foote, In Free Radicals in Biology, W. A. Pryor Ed, Academic Press, New York, 1976, Vol. II, Chap 3.

21. C. S. Foote, In 'Oxygen and Oxy Radicals in Chemistry and Biology' M. A. J. Rodgers, E. L. Powers Ed., Academic Press, New York, 1981, p 425.

22. C. S. Foote, In 'Porphyrin Localization and Treatment of Tumors' D. R. Doiron, C. J. Gromer, A. R. Liss Ed., Academic Press, New York, 1984, p 3.

23. M. J. Peak, J. G. Peak, C. S. Foote, N. L. Krinsky, *J. Photochem.*, 1984, **25**, 309.

24. M. Korycka-Dohl, T. Richardson, *Biochem. Biophys. Acta*, 1980, **610**, 229.

25. P. Kang, C. S. Foote, *J. Am. Chem. Soc.*, 2002, **124**, 4865.

26. http://www. Chem.ucla.edu/dept/organic/ CSF_Brochure.html.

27. C. S. Foote, *Photochem. Photobiol.*, 1991, **54**, 659.

28. M. C. DeRosa, R. J. Crutchley, *Coord. Chem. Rev.*, 2002, **233-234**, 351.

29. J. D. Coyle, In 'Photochemistry in Organic Synthesis' Royal Society of Chemistry, Burlington House, 1986, Spl. Publ. No. 57, p 191.

30. S. Hecht, J. M. J. Frechet, *J. Am. Chem.* Soc., 2001, **123**, 6959.

31. M. Benaglia, T. Danelli, F. Fabris, D. Sperandis, G. Pozzi, *Org. Lett.*, 2002, **4**, 4229.

32. C. Pierlot, V. Nardello, J. Schrive, C. Mabille, J. Barbillat, B. Sombret, J. M. Aubry, *J. Org. Chem.*, 2002, **67**, 2418.

33. E. L. Clennan, *Tetrahedron*, 2000, **50**, 9151.

34. M. Orfanoponlos, *Tetrahedron*, 2000, **56**, 1959.

35. B. Halliwell, M. C. John, Free Radicals in Biology and Medicines, Second Ed., Clarwndon Press, Oxford, 1982.

36. P. A. Thomas, Devasagayam, J. P. Kamat, *Indian. J. Expl. Biol.*, 2002, **40**, 693.

37. H. D. Rabinowitch, I. Fridovich, *Photochem. Photobiol.*, 1983, **37**, 679.

38. U. Takahama, M. Nishimura, *Plant Cell Physiol.*, 1975, **16**, 737.

39. M. P. percival, A. D. Dodge, *Plant Sci. Letters*, 1983, **29**, 255.

40. M. P. percival, A. D. Dodge, *Z. Naturforsch*, 1984, **39c**, 482.

41. A. Dorsman, O. Elgersma, G. Meijer, L. Steuten, *Photochem. Photobiol.*, 1977, **26**, 533.

42. J. L. P. Van Oorschot, *Weed Res.*, 1974, **14**. 75.

43. R. R. Wise, J. R. McWilliam, A. W. Naylor, *Plant Cell Environ.*, 1983, **6**, 525.

44. K. E. Pallet, A. D. Dodge, *J. Exp. Botany*, 1980, **31**, 1051.

45. A. D. Dodge, *Prog. Pestic. Biochem. Tox.*,1983, **3**, 163.

46. J. P. Knox, A. D. Dodge, *Plant Cell Environ.*, 1985, **8**, 19.

47. M. A. Pathak, P. C. Joshi, *Biochem. Biophys. Acta*, 1984, **789**, 115.

48. F. Wilkinson, J. G. Brummer, *J. Phys. Chem. Ref. Data,* 1981, **10**, 809.

49. J. G. Parker, W. D. Stanbro, *J. Photochem.*, 1984, **25**, 545.

50. N. I. Krinsky, *Singlet Oxygen,* In H. H. Wasserman Ed., Academic Press. 1979, Vol. 40. pp. 597

51. A. A. Frimer, *Chem. Rev.*, 1979, **79**, 359.

52. M. Perin, W. Adam, *Angew. Chem. Int. Ed. Engl.*, 1996, **35**, 477.

53. (a) C. G. Daughton, T. A. Ternes, *Environ. Health Pers.,* 1999, 107; (b) A. L. Boreen, W. A. Arnold, K. McNeil, *Aquat. Sci.*, 2003, **65**, 320.

54. O. C. Zafiriou, J. J. Dubien, R. G. Zepp, R. G. Zika, *Environ. Sci. Technol.*, 1984, **18**, 358A.

55. F. Urbach, in "The Science of Photomedicine", J. D. Regan, J. A. Parrish Eds., Plenum press, New York 1982, p 261.

56. L. H. Kligman, A. M. kligman, *Photodermatol.*, 1986, **3**, 215.

57. I. Abramov, in "The medical and biological effects of light", R. J. Wurtman, M. J. Baum and J. T. Potts Ed., Annals of New York Academy of Sciences, The New York academy of science, New York, 1985, vol. 453, p 365,.

58. M. B. McElory, R. J. Salawitch, *Science*, 1989, **243**, 763.

59. G. M. J. Beijersbergen Van Henegouwen, In B. Testa U. Meyer Ed., Advances in drug Research, Academic, New York and London, 1979, **29**, p 79.

60. D. R. Bicker, In Harrison's Principles of Internal medicine, J. D. Wilson, E. Braunwaid, K. J. Isselbacher et al Ed., McGraw-Hill, New York, 1991, p 338.

61. J. A. Parrish, H. A. D. White, M. A. Pathak, In Dermatology in General Medicine, T. B. Fitzpoatrick, A. Z. Eisen, K. Wolff, I. M. Freedberg, K. F. Austen Ed., McGraw-Hill Book Company, New York, 1979, p 942.

62. W. L. Morison, in "The medical and biological effects of light", R. J. Wurtman, M. J. Baum and J. T. Potts eds., Annals of New York Academy of Sciences, The New York academy of science, New York, 1985, vol. 453, p 105.

63. P. J. G. Cornelissen, G. M. J. Beijersbergen van Henegouwen, G. R. Mohn, *Photochem. Photobiol.*, 1980, **32**, 653.

64. A. Bakri, G. M. J. Beijersbergen van Henegouwen, H. deVries, *Pharm. Weekbl. Sci.*, 1988, **10**, 122.

65. S. A. Schoonderwoerd, G. M. J. Beijersbergen van Henegouwen, S. Belkum, *Photochem. Photobiol.*, 1989, **50**, 659.

66. S. A. Schoonderwoerd, G. M. J. Beijersbergen van Henegouwen, *Photochem Photobiol.*, 1887, **46**, 501.

67. J. A. Parrish, T. B. Fitzpatrick, L. Tanenbaum, *N. Engl. J. Med.*, 1974, **291**, 1207.

68. F. Dall'Acqua, S. Caffieri, G. Rodighiero, In ' Primary Photoprocess in Biology and Medicine' R. V. Bensasson, G. Jori, E. J. Land, T. G. Truscott Ed., Plenum Press New York, 1985, p 277.

69. J. A. Parrish, R. S. stern, M. A. Pathak, T. B. Fitzpatrick, In ' The Science of Photomedicine' J. D. regan, J. A. Parrish Ed., Plenum Press New York, 1982, p 595.

70. B. Honig, W. L. Morison, D. Karp, *J. Am. Acad. Dermatol.*, 1994, **31**, 775.

71. G. Jori, in: R. V. Bensasson, G. Jori, E. J. Land and T. G. Truscott Ed. Plenum Press, New York, 1985, p 381.

72. J. Moan, *Photochem. Photobiol.*, 1986, **43**, 681.

73. G. I. Stables, D. V. Ash, *cancer Treatment reviews*, 1995, **21**, 311.

74. G. M. J. Beijersbergen van Henegouwen, E. T. Wijn, S. A. Schoonderwoerd, F. Dall' Acqua, *J. Photochem. Photobiol. B: Biol.*, 1989, **3**, 631.

75. S. A. Schoonderwoerd, G. M. J. Beijersbergen van Henegouwen, C. C. M. Persons, S. caffieri, F. Dall' Acqua, *J. Photochem. Photobiol. B: Biol.*, 1991, **10**, 257.

76. H. H. Tonnesen, in: Photostability of Drugs and Drug Formulations, H. H. Tonnesen Ed., Taylor and Francis, London, 1996.

77. W. H. Horspool, P. S. Song, J. V. Greenhill, CRC Handbook of Organic Photochemistry and Photobiology, CRC press, Boca Raton, 1995, p 83.

78. S. Hashiba, M. Tatsuzawa, A. *Eijma, Eisei Shikensho Hokoku*, 1979, **97**, 73 through Chem. Abstr., 1980, **93**, 16854n.

79. T. M. Chen, A. D. Sill, C. L. Housmyer, *J.Pharm. Biomed. Anal.*, 1986, **4**, 533.

80. V. J. Hemmens, D. E. Moore, *Photochem. Photobiol.*, 1986, **43**, 257.

81. F. Vargas, C. Rivas, M. A. Miranda, F. Bosca, *Pharmazie*, 1991, **46**, 767.

82. D. E. Moore, P. P. Chappuis, *Photochem. Photobiol.*, 1988, **47**, 173.

83. G. Condorelli, G. De Guidi, G. Giufrida, L. L. Costanzo, *Coord. Chem. Rev.*, 1993, **125**, 115.

84. D. Budac, P. Wan, *J. Photochem. Photobiol.*, 1992, **67**, 135.

85. K. Reszka, C. F. Chignel, *Photochem. Photobiol.*, 1983, **38**, 281.

86. F. Bosca, M. A. Miranda, L. Vano, F. Vargas, *J. Photochem. Photobiol. A: Chem.*, 1990, **54**, 131.

87. J. V. Castell, M. J. Gomez-Lechon, M. A. Miranda, I. M. Morrea, *Photochem. Photobiol.*, 1987, **46**, 991.

88. J. V. Castell, M. J. Gomez-Lechon, M. A. Miranda, *J.Photochem. Photobiol.*, B: Biol., 1992, **13**, 71.

89. S. Monti, S. Sortino, G. De Guidi, G. Marconi, *J. Chem. Soc. Faraday Trans.*, 1997, **93**, 2269.

90. F. Bosca, M. A. Miranda, G. Carganico, D. Mauleon, *Photochem. Photobiol.*, 1994, **60**, 96.

91. J. V. Castel, M. J. Gomez-Lechon, C. Grassa, L. A. Martinez, M. A. Miranda, P. Tarrega, *Photochem. Photobiol.*, 1994, **59**, 35.

92. S. Navaratnam, J. L. Hughes, B. J. Parsons, G. O. Philips, *Photochem. Photobiol.*, 1985, **41**, 375.

93. F. Bosca, M. A. Miranda, F. Vargas, *J. Pharm. Sci.*, 1992, **81**, 181.

94. L. Gu, H. S. Chiang, D. Johnson, *Int. J. Pharm.*, 1988, **41**, 105.

95. A. C. Weedon, D. F. Wang, *J. Photochem. Photobiol., A: Chem.*, 1991, **61**, 27.

96. R. Dabestani, R. H. Sik, D. G. Davis, G. Dubay, C. F. Chignell, *Photochem. Photobiol.*, 1993, **58**, 367.

97. A. B. Wu, H. W. Cheng, C. M. Hu, F. A. Chen, T. C. Chou, C. Y. Chen, *Tetrahedron Lett.*, 1997, **38**, 621.

98. D. E. Moore, S. Roberts-Thomson, D. Zhen, C. C. Duke, *Photochem. Photobiol.*, 1990, **52**, 685.

99. J. Philip, D. H. Szulczewski, *J. Pharm. Sci.*, 1973, **62**, 1479.

100. F. Vargas, C. Rivas, R. Machado, Z. Sarabia, *J. Pharm. Sci.*, 1993, **82**, 371.

101. J. V. Castell, M. J. Gomez-Lechon, V. Mirabet, M. A. Miranda, I. M. Morea, *J. Pharm. Sci.*, 1987, **76**, 374.

102. J. Jensen, C. Cornett, C. E. Olsen, J. Tjoernelund, S. H. Hansen, *Int. J. Pharm.*, 1992, **88**, 177.

103. J. Reisch, L. Iranshani, N. Ekiz-Guecer, *Liebigs Ann. Chem.*, 1992, 1199.

104. C. T. Lin, P. Perrier, G. G. Clay, P. A. Sutton, S. R. Byrn, *J. Org. Chem.*, 1982, **47**, 2978.

105. K. Shaffner, M. Demuth, in: Rearangements in the ground and excited state, P. De Mayo Ed., Academic Press, New York, 1980, Vol 3, p 281.

106. T. Hidaka, S. Huruumi, S. Tamaki, M. Shiraishi, H. Minato, *Yakagaku Zasshi*, 1980, **100**, 72.

107. K. Thoma, R. Kerker, C. Weissbach, *Pharm. Ind.*, 1987, **49**, 961.

108. K. Jochym, H. Barton, J. Bojarski, *Pharmazie*, 1988, **43**, 623.

109. H. Barton, J. Bojarski, J. Mokrosz, *Tetrahedron Lett.*, 1982, **23**, 2133.

110. H. Barton, Z. Alexandra, J. Bojarski, W. Welna, *Pharmazie*, 1983, 38, 268.

111. H. Barton, J. Bojarski, A. Zurowska, *Arch. Pharm. (Weinheim, Ger.)*, 1986, **319**, 457.

112. J. Mokrosz, A. Zurowska, J. Bojarski, *Pharmazie*, 1982, **37**, 832.

113. H. Barton, J. Mokrosz, J. Bojarski, M. Klimezak, *Pharmazie*, 1980, **35**, 155.

114. M. H. Paluchowska, H. J. Bojarski, *Arch. Pharm. (Weinhem)*, 1988, **321**, 343.

115. D. E. Moore, *J. Pharm. Sci.*, 1977, **66**, 1282.

116. R. Andersin, J. Ovaskainen, S. Kaltia, *J. Pharm. Biomed. Anal.*, 1994, **12**, 165.

117. R. Andersin, M, Mwsilaakso, *J. Pharm. Biomed. Anal.*, 1995, **13**, 667.

118. H. J. Roth, M. Adomeit, *Arch. Pharm. (Weinheim)*, 1973, **306**, 889.

119. R. W. Busker, G. M. J. Beijersbergen van Henegouwen, B. M. C. Kwee, H. M. Winkens, *Int. J. Pharm.*, 1987, **36**, 113.

120. R. S. Givens, J. Gingrich, S. Mecklemburg, *Int. J. Pharm.*, 1986, **29**, 67.

121. A. Bakri, G. M. J. Beijersbergen van Henegouwen, J. L. Chanal, *Photodermatology*, 1985, **2**, 205.

122. P. J. G. Cornelissen, G. M. J. Beijersbergen van Henegouwen, K. W. Gerritsma, *Int. J. Pharm.*, 1979, **3**, 205.

123. J. Reisch, N. Ekiz-Guecer, G. Tewes, *Liebigs Ann. Chem.*, 1992, 69.

124. P. J. G. Cornelissen, G. M. J. Beijersbergen van Henegouwen, *Pharm. Weekbad, Sci. Ed.*, 1980, **2**, 39.

125. P. Pietta, A. Rava, P. A. Biondi, *J. Chromatogr.*, 1981, **210**, 516.

126. W. A. Al-Turk, I. A. Majeed, W. J. Murray, D. W. Newton, S. Othman, *Int. J. Pharm.*, 1988, **41**, 227.

127. G. S. Sadana, A. B. Ghogare, *Int. J. Pharm.*, 1991, **70**, 195.

128. M. C. Bonferoni, G. Mellerio, P. Giunchedi, C. Caramela, U. Conte, *Int. J. Pharm.*. 1992, **87**, 133.

129. L. J. Nunez-Vergara, C. Sunkel, J. A. Squella, *J. Pharm. Sci.*, 1994, **83**, 502.

130. A. L. Zanocco, L. Diaz, M. Lopez, L. J. Nunez-Vergara, J. A. Squella, , *J. Pharm. Sci.*, 1992, **81**, 920.

131. M. S. Suleiman, M. E. Abdulhameed, N. M. Najib, H. Y. Muti, *Int. J. Pharm.*, 1989, **50**, 71.

132. R. Karlicek, V. Klimesova, *Cesk. Farm.*, 1977, **26**, 413.

133. N .Jamil, H. Afrozrizvi, I. Ahmed, A. Jazbeg, *Pharmazie*, 1983, **38**, 467.

134. G. E. Wright, T. Y. Tang, *J. Pharm. Sci.*, 1972, **61**, 299.

135. F. Golpashin, B. Weiss, H. Durr, *Arch. Pharm. (Weinheim)*, 1984, **317**, 906.

136. H. C. Chiang, S. J. S. Chen, L. C. Yeh, *J. Taiwan Pharm. Assoc.*, 1976, **28**, 36.

137. J. Pawlaczyck, W. Turowska, *Acta Pol. Pharm.*, 1976, **33**, 505.

138. J. Reisch, D. H. Niemeyer, *Arch. Pharm. (Weinheim),* 1972, **305**, 135.

139. A. K. Davies, J. F. McKellar, G. O. Philips, A. G. Reid, *J. Chem. Soc. Perkin Trans. 2*, 1979, 369.

140. D. E. Moore, M. P. Falon, C. D. Burt, *Int. J. Pharm.*, 1983, **14**, 133.

141. A. S. W. Li, C. F. Chignell, R. D. Hall, *Photochem. Photobiol.*, 1987, **46**, 379.

142. J. Reisch, K. G. Weidmann, , *Arch. Pharm. (Weinheim)*, 1971, **304**, 911.

143. S. I. M. Mubarak, J. B. Standford, J. K. Sudgen, *Pharm. Acta Helv.*, 1982, **57**, 226.

144. S. I. M. Mubarak, J. B. Standford, J. K. Sudgen, *Pharm. Acta Helv.*, 1983, **58**, 343.

145. A. B. Narbutt-Mering, W. Weglowska, *Acta Pol Pharm.*, 1980, **37**, 301.

146. D. E. Moore, B. J. Wilkins, *Radiat. Phys. Chem.,* 1990, **36**, 547.

147. B. J. Wilkins, G. J. Gainsford, D. E. Moore, *J. Chem. Soc. Perkin Trans. 1,* 1987, 1817.

148. K. H. Pfoertner, J. J. Daly, *Helv. Chim. Acta*, 1987, **70**, 171.

149. R. Godfrey, R. Edwards, *J. Pharm. Sci.*, 1991, **80**, 212.

150. I. Ninomiya, O.Yamamoto, *Heterocycles*, 1976, **4**, 475.

151. J. J. Bergh, J. C. Breytenbach, P. L. Wessels, *J. Pharm. Sci.*, 1989, **78**, 348.

152. B. Quintero, M. A. Miranda, *Ars Pharmaceutica,* 2000, **41**, 27.

153. A. Albini, S. Monti, *Chem Soc Rev.*, 2003, **32**, 238.

154. W. A. Laurie, D. McHale, K. Saag, *Tetrahedron*, 1986, **42**, 3711.

155. L. Pasteur, *Compt. Rend.*, 1853, **37**, 110.

156. A. Brossi, W. P. Gessner, C. D. Hufford, J. K. Baker, F. Homo, P. Millet, I. Landau, *FEBS Lett.*, 1987, **223**, 77.

157. S. Kristensen, A. L. Grislingaas, J. V. Greenhill, T. Skjetne, J. Karlsen, H. H. Tonnesen, *Int. J. Pharm.*,1993, **100**, 15.

158. J. A. Owoyale, *Int. J. Pharm.*, 1989, **56**, 213.

159. H. H. Tonnesen, A. L. Grislingaas, S. O. Woo, J. Karlsen, *Int. J. Pharm.*, 1988, **43**, 215.

160. K. Nord, J. Karlsen, H. H. Tonnesen, *Int. J. Pharm.*, 1991, **72**, 11.

161. J. A. Owoyale, *Int. J. Pharm.*, 1989, **52**, 179.

162. J. J. Aaron, J. Fidanza, *Talanta*, 1982, **29**, 238.

163. H. H. Tonnesen, A. L. Grislingaas, *Int. J. Pharm.*, 1990, **60**, 157.

164. J. K. Horton, M. F. G. Stevenes, *J. Pharma. Pharmacol.*, 1981, **33**, 808.

165. A. Fredenhagen, U. Sequin, *Helv. Chim. Acta*, 1985, **68**, 391.

166. V. J. hemmens, D. E. Moore, *J. Chem. Soc. Perkin Trans. 2*, 1984, 209.

167. F. Vargas, K. Carbonell, M. Camacho, *Pharmazie,* 2003, **58**, 315.

168. S. Sortino, G. Marconi, G. Condorelli, *Chem Commun.*, 2001, 1226.

169. S. Sortino, S. Giuffrida, G. De Guidi, R. Chillemi, S. Petralia, G. Marconi, G. Condorelli, S. Sciuto, *Photochem. Photobiol.*, 2001, **73**, 6.

170. R. J. Frith, G. Phillipou, *J. Chromatogr.*, 1986, **367**, 260.

171. D. W. Mendenhall, H. Kobayashi, F. M. L. Shih, L. A. Sternson, T. Higuchi, C. Fabian, *Clin. Chem.*, 1978, 1518.

172. T. D. Doyle, W. R. Benson, N. Filipescu, *J. Am. Chem. Soc.*, 1976, **98**, 3262.

173. P. Hugelshofer, J. Kalvoda, K. Schaffner, *Helv. Chim. Acta*, 1960, **43**, 1322.

174. T. D. Doyle, W. R. Benson, N. Filipescu, *Photochem. Photobiol.*, 1978, **27**, 3.

175. A. G. J. Sedee, G. M. J. Beijersbergen van Henegouwen, *Arch. Pharm. (Weinheim)*, 1985, **318**, 111.

176. A. G. J. Sedee, G. M. J. Beijersbergen van Henegouwen, N. J. de Mol, G. Lodder, *Chem. Biol. Interact.*, 1984, **51**, 357.

177. J. Reisch, N. Ekiz-Guecer, G. Tewes, G. Henkel, *Liebigs Ann. Chem.*, 1989, 595.

178. J. Reisch, N. Ekiz-Guecer, M. Takacs, *Arch. Pharm. (Weinheim)*, 1989, **322**, 173.

179. J. Reisch, J. Zappel, A. R. R. Akinepalli, G. Henkel, *Pharm. Acta Helv.*, 1994, **69**, 97.

180. J. Reisch, J. Zappel, G. Henkel, N. Ekiz-Guecer, *Monatsh. Chem.*, 1993, **124**, 1169.

181. J. Reisch, N. Ekiz Guecer, M. Takacs, G. Henkel, *Liebigs Ann. Chem.*, 1989, 595.

182. L. Zanocco, G. S. Gunther, E. M. Lemp, JR de la Fuente, N. U. Pizarro, *Photochem. Photobiol.*, 1998, **68**, 487.

183. F. Vargas, H. Mendez *Die Pharmazie,* 1999, **54**, 920.

184. C. W. Brown, J. S. Deng, *J. Toxicol. Clin. Toxicol.*, 1995, **33**, 729.

185. L. Mirossay, A. Mirossay, E. Kocisova, I. Radvakova, P. Miskovsky, J. Mojzis, *Physiol. Res.*, 1999, **48**, 135.

186. Fu Yulan, C. Sheu, T. Fujita, C. S. Foote, *Photochem Photobiol.,* 1996, **63**, 615.

187. S. Criado, D. Mártire, P. Allegretti, J. Furlong, S. Bertolotti, E. La Falce, N. García, *Pharmazie,* 2003, **58**, 551.

188. U. Girreser, T. W. Hermann, K. Piotrowska, *Arch Pharm (Weinheim).*, 2003, **336**, 401.

189. N. Canudas, C. Contreras, *Pharmazie,* 2002, **57**, 405.

190. E. D. Morgan, A. P. Jarvis, G. R. Jones, *ARKIVOC*, 2000, **1**, 312.

191. S. Walia, S. K. Kulshrestha and S. K. Mukherjee, *Tetrahedron,* 1986, **42**, 4817.

192. Wu Shuping, J. Zhiqin, Li Heting, Y. Li, Z. Daixun, *Molecules,* 2001, **6**, 52.

193. T. Matsuura, H. Matsushima and R. Nakashima, *Tetrahedron,* 1970, **26**, 435.

194. K. -S. Ngo, G. D. Brown, *Tertahedron,* 1999, **55**, 15109.

195. T. Hata, K. tanaka, S. Katsumura, *Tetrahedron Lett.*, 1999, **40**, 1731.

196. M. Sakai, M. Sasaki, K. Tanino, M. Miyashita, *Tetrahedron Lett.*, 2002, **43**, 1705.

197. W. Adam, E. Kades, X. Wang, *Tetrahedron Lett.*, 1990, **31**, 2259.

198. M. M. Hashemi, S. Balalaie, *J. Chem. Res., (s)* 1996, 170.

199. Z. –F. Tao, X. Qian, M. Fan, *Tetrahedron* 1997, **53**, 13329.

200. C. A. Mateo, A. Urrutia, J. G. Rodriguez, I. Fonseca, F. H. Cano, *J. Org. Chem.*, 1996, **61**, 810.

201. M. Ihara, K. Noguchi, T. Ohsawa, K. Fukumoto, T. Kametani, *J. Org. Chem.* 1983, 48, 3150.

202. M. Nagakawa, Y. Yokoyama, S. kato, T. Hino, *Tetrahedron* 1985, 41, 2125.

203. M. Nagakawa, S. kato, S. Kataoka, S. Kodato, H. Watanabe, H. Okajima, T. Hino, B. Witkop, *Chem. Pharm. Bull.* 1981, 29, 1013.

204. M. Nagakawa, S. kato, H. Fukazawa, Y. Hasegawa, J. Miyazawa, T. Hino, *Tetrahedron Lett.* 1985, 26, 5871.

Chapter 2

Photochemical Studies on Acyclovir and Phenazopyridine Hydrochloride

Introduction

The treatment of disease requires the use of either systemic or topical medication during certain period of time. Frequently the treatment coincides with exposure to electromagnetic radiations, coming from different types of sources (sunlight in works made outdoor and intense artificial radiation used in specific works etc.). This coincidence may lead to photobiological effects such as drug photosensitization, phototoxicity, photodegradation etc. The molecular mechanism of biological photosensitization induced by drugs and their phototoxicity is receiving increasing attention.[1-5] With regard to the mechanistic pathways, it is accepted basically the four paths as main routes for phototoxic reactions,[5] namely ***singlet oxygen formation and its reaction with drug, radical formation, covalent photobinding to biomolecules and photoproducts in decomposition reaction.***

Studies on both, the drug induced singlet oxygen formation and its reaction with drug and photodegradation of drugs are relevant to drug development process, because the photoproducts may have biological effects different from those of parent compounds. These studies are of high significance in current medicinal chemistry as this may explain, at least partially, phototoxicity mechanism.

With this interest herein we have investigated:

[A]. 1. Photooxidation of acyclovir in aqueous solution.

[A].2. Photooxidation of acyclovir by thermally generated triplet excited ketone from 1,2-dioxetane and its comparison with type I and type II photosensitizers.

[B] Photochemistry of Phenazopyridine hydrochloride.

Section [A]

Photooxidation Studies on Acyclovir

[A]. 1. Photooxidation of acyclovir in aqueous solution

Acyclovir (9-[(2-hydroxyethoxy) methyl] guanine) (**Ac**, **1**) is an antiviral drug used for the treatment of herpes encephalitis caused by herpes simplex virus or varicella zoster infections. Acyclovir is mainly used to treat chickenpox, shingles, and the symptoms of herpes virus infections of the genitals, lips, mouth, skin, and brain. The medicine does not cure the infections, but it relieves the discomfort and speeds healing of sores, when they are present.[6]

Although acyclovir has a large therapeutic index and usually well-tolerated, acute renal failure and neurotoxicity are two important potential adverse effect of this drug.[7,8] It is metabolized, probably by alcohol dehydrogenase and aldehyde dehydrogenase, to 9-carboxymethoxymethyl guanine and to a smaller extent to 8-hydroxy-9-(2-hydroxyethoxymethyl) guanine (8-OH-Acyclovir).[9] Acyclovir is structurally related to deoxyguanosine and with photoactivatable chromophores and electron rich heterocyclic ring it is prone to photochemical transformation, including reaction with the electrophilic singlet oxygen.

Experimental

Chemicals

All chemicals used were of analytical grade. Acyclovir was extracted, from the commercial medicament Acivir (Cipla Limited, Mumbai, India) with a soxhlet extractor using methanol as the solvent and recrystallized from the same

solvent. Melting point, ^{1}H-NMR and co-TLC with the authentic pure sample determined the purity of acyclovir.

Apparatus

Irradiations were carried out in a photoreactor equipped with medium pressure mercury vapour lamp (Philips, 450 W) inserted in a water-cooled immersion well with continuous supply of water. The incident photon flux of the irradiation setup was 9.78 × 10^{-7}einstein/min as determined by using ferrioxalate actinometry.[10] IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RX1. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 spectrometer using $SiMe_4$ as internal standard. EIMS and FAB-mass spectra were recorded on VG-ZAB-HS and Jeol SX 102/DA-6000 mass spectrometers at 10 KV accelerating voltage spectrometer using *m*-nitrobenzyl alcohol (NBA) matrix and argon as FAB gas, respectively. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 eV ionization voltage.

Photooxidation procedure

Irradiation of an aqueous solution of **Ac** (5 mM) in 50 mM phosphate buffer (pH 7) was carried out in, the absence and in presence of rose bengal (0.1mM) as sensitizer, with medium pressure mercury vapour lamp. The solution was continuously stirred during photolysis and the temperature of the solution was kept at 15°C during irradiation by cooling with a water streamer immersed in the solution. A corex filter transmitting above 270 nm was used for photolysis

of **Ac**. Progress of the reaction was monitored by thin layer chromatography. After 6 h of irradiation, removal of solvent in a rotary evaporator (30°C) and silica gel column chromatography (chloroform : methanol) of the photolysate yielded compounds **2**, **3** and **4** as products. The above photoreaction was also examined in deuterium oxide and in the additive presence of sodium azide (1.0 mM), for establishing the involvement of singlet oxygen in this photoreaction.

Potassium Ferrioxalate Actinometry

The amount of ferrous produced is measured via spectrophotometric determination of its 1, 10-phenanthroline complex at 510 nm. Ferric apparently forms only a weak complex with 1, 10-phenanthroline, and this complex is transparent at 510 nm.

$$2Fe^{3+} + C_2O_4^{2-} \xrightarrow{h\nu} 2Fe^{2+} + 2CO_2$$

Preparation of Actinometer solution

1. $Fe_2(SO_4)_3$ solution (0.2 mol/L) was titrated with standardized EDTA using 0.2 gm salicylic acid/100 mL solution as indicator and buffered with 0.3 gm glycine/100 mL solution, to a pH of 3-4.
2. A 100 mL solution of $K_2C_2O_4$ was prepared in such a way that its molarity is six times that of the $Fe_2(SO_4)_3$ solution (approximately 1.2 mol/L $K_2C_2O_4$).
3. When actinometer solution was needed, 5mL of $Fe_2(SO_4)_3$ solution and 5 mL of $K_2C_2O_4$ solution was pipetted into a 100 mL volume flask and diluted to the mark with water.

Intensity Measurement

a) A volume of $K_3Fe(C_2O_4)_3$ solution equal to that of the samples to be irradiated was pipetted into the reaction vessel.

b) Irradiated for an appropriate period of time.

c) Irradiated solution was mixed thoroughly and an aliquot (1mL) of the actinometer was pipetted into a 10 mL volumetric flask.

d) 2 mL of 0.2 % 1,10-phenanthroline solution was added to this.

e) A volume of buffer equal to one half of the aliquot of the actinometer taken was added and diluted to the mark with water.

f) A blank was prepared by following the steps c-e with a non irradiated volume of actinometer equal to the aliquot of irradiated sample withdrawn.

g) The absorbances of the solutions 'e' and 'f' were measured vs. water at 510 nm and difference was taken.

Calculation of light intensity: using the absorbance obtained the light intensity was calculated from the following formula.

$$I \text{ (einsteins/min)} = \frac{AV_2V_3}{\varepsilon d \varphi_\lambda t V_1}$$

Where

A Absorbance (at 510 nm) of the irradiated actinometer solution corrected for absorption of blank. (0.831)

d Path length of the absorption cell used in measurement of A (1cm).

ε Extinction coefficient of ferrous 1, 10-phenanthroline complex at 510 nm (1.11×10^4 L/mol/cm).

φ_λ Quantum yield of ferrous production at wavelength of light used. (1.03)

V_1 Volume (in milliliters) of irradiated actinometer solution withdrawn.

V_2 Volume (in liters) of actinometer irradiated. (0.20 L)

V_3 Volume (in milliliters) of volumetric flask used for dilution of irradiated aliquot (10 mL).

t irradiated time (150 min).

Characterization of products

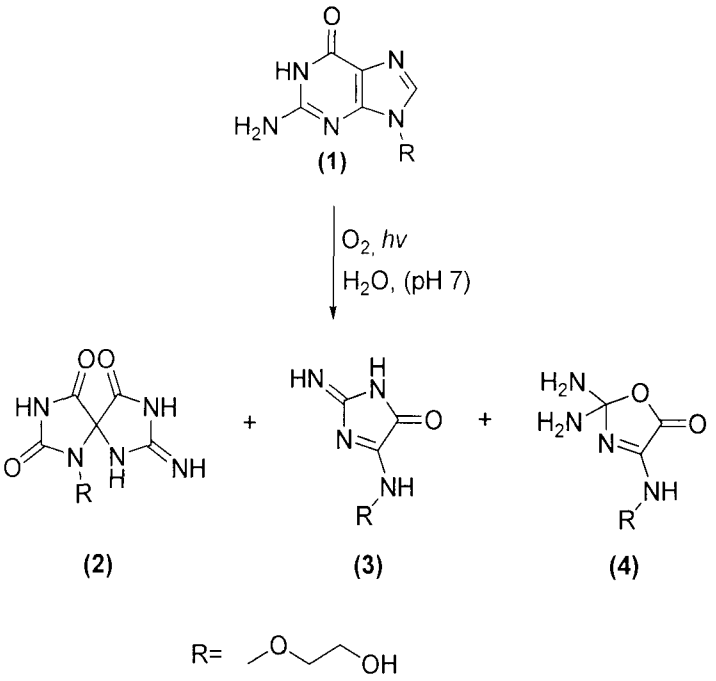
(2-Hydroxyethoxy) methyl spiroiminodihydantion (2): yield: 85.50 mg (38%); HRMS calcd. for (M^+) $C_8N_5O_5H_{11}$ 257.2054, found 257.2057; IR (KBr) : 3490, 3475, 3365, 3320, 1800, 1760, 1735, 1700, 1150 cm^{-1}; ^{1}H-NMR (DMSO, δ, ppm): 8.43, 8.38, 8.35, 8.27 (4H, NH), 5.24 (d, 2H), 3.71 (t, 2H), 3.56 (t, 2H); ^{13}C-NMR (DMSO, δ, ppm) : 181.9 (C-8), 172.5 (C-4), 171.3 (C-6), 165.8 (C-2), 85.1 (C-5), 69.7, 67.9, 61.1; FAB-MS m/z: 258 $[C_8H_{11}N_5O_5+H]^+$, 184 $[C_5H_5N_5O_3+H]^+$.

4-[(2-Hydroxyethoxy) methyl amino]-2-imino-1,2-dihydroimidazole-5-one (3): yield: 20.25 mg (9%); HRMS calcd. for (M^+) $C_6H_{10}N_4O_3$ 186.1700, found 186.1698; IR (KBr): 3240, 1734, 1645, 1528, 1155 cm^{-1}; ^{1}H-NMR (DMSO, δ, ppm): 9.08 (2-NH), 8.90 (3-NH), 9.31 (5-NH), 4.73 (d, 2H), 3.70 (t, 2H), 3.56 (t, 2H); ^{13}C-NMR (DMSO, δ, ppm) : 184.8 (C-2), 176.7 (C-4), 166.5 (C-5), 69.8, 69.2, 61.1; FAB-MS m/z: 187 $[C_6H_{10}N_4O_3+H]^+$, 113 $[C_3H_4N_4O+H]^+$.

2,2-Diamino-4-[(2-hydroxyethoxy)methyl) amino]-5-[2H]-oxazolone (4): yield: 24.75 mg (11%); HRMS calcd. For (M^+) $C_6H_{12}N_4O_4$ 204.1840, found, 204.1845; IR (KBr) : 3350, 2945, 1780, 1735, 1659, 1490, 1160 cm^{-1}; ^{1}H-NMR (DMSO, δ, ppm): 7.58 (NH_2, 4H), 8.20 (4-NH), 4.73 (d, 2H), 3.70 (t, 2H), 3.56 (t, 2H); ^{13}C-NMR (DMSO, δ, ppm) : 166.3 (C-2), 156.1 (C-4), 160.1 (C-5), 69.9, 69.8, 61.1; FAB-MS m/z: 205 $[C_6H_{12}N_4O_4+H]^+$, 131 $[C_3H_6N_4O_2+H]^+$, 161 $[C_6H_{12}N_4O_4-CO_2+H]^+$, 87 $[C_3H_6N_4O_2-CO_2+H]^+$.

Results and discussion

Irradiation of air saturated aqueous solution of **Ac** (**1**) in pH 7 phosphate buffer with corex filtered light followed by purification of crude product by silica gel column chromatography, afforded three major products, which were identified by their spectral studies as: (2-hydroxyethoxy) methyl spiroiminodihydantion (**2**), 4-[(2-hydroxyethoxy) methyl amino]-2-imino-1,2-dihydroimidazole-5-one (**3**), and 2,2-diamino-4-[(2-hydroxyethoxy)methyl) amino]-5-[2H]-oxazolone (**4**) (Scheme 2A.1). The study was supplemented by irradiation in the presence of rose bengal, whereby same products were obtained, with considerably greater conversion in a much shorter time. When rose bengal was replaced with silica bound rose bengal[11] the rate of photooxidation of **Ac** was slower but it contributed to a clean workup.



Scheme 2A.1

A brown colouration was observed for compound **4** after spraying the silica gel TLC plates with the hydroxylamine iron (III) chloride, suggesting the presence of lactone moiety in compound **4**. The photomediated transformation of **Ac** involves singlet oxygen as evidenced from following observations: (1) No loss of substrate was observed when oxygen was excluded from medium; (2) Destruction of substrate was quenched in presence of sodium azide, a singlet oxygen quencher; (3) Loss of substrate was accelerated in D_2O, a well known

singlet oxygen life time promoter; (4) The enhanced degradation in D_2O was also inhibited by sodium azide.

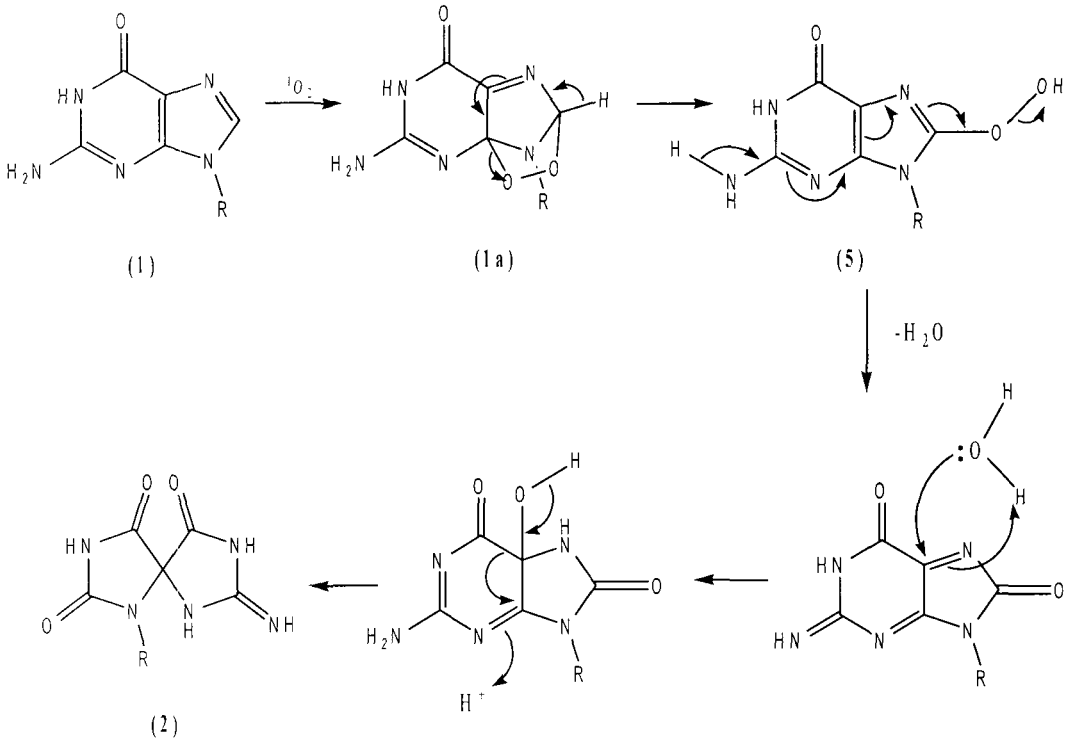
A comparison of ^{1}H-NMR and ^{13}C-NMR spectra of **Ac** and those of photoproducts **2**, **3** and **4** did not show any significant change in the chemical shifts of protons and carbon atoms of side chain. The slight up field shift observed for methylene protons of side chain with respect to **Ac** may be explained by loss of aromaticity of heterocyclic ring. The lack of H-8 resonance signal (δ 8.05) in the low field region of the ^{1}H-NMR spectrum indicates that purine ring of the substrate has been modified. In the ^{13}C-NMR spectrum of compound **2** signal at δ 181.9 was assigned to imine type carbon C-8 and signals at δ 165.8, 172.5 and 171.3 ppm were attributed to carbonyl carbon at C-2, C-4 and C-6 respectively. A significant feature of ^{13}C-NMR spectrum is the appearance of a new resonance signal at δ 85.1 ppm, which was assigned to the quaternary carbon in the spiro ring in comparison with the spectra of related spirohydantoins.[12,13] The presence of hydantoin ring in product **2** is evidenced from its infrared spectrum, which showed a sharp absorption band at 1800 cm^{-1} along with three intense absorption bands in the region 1780-1700 cm^{-1}.[14] Inspection of the low field region of the ^{13}C-NMR spectra of both the photoproducts, **3** and **4**, revealed the loss of two carbon atoms in the starting compound. Resonance signals at δ 8.20 for **4** and δ 9.31 for **3**, exchangeable with D_2O, were assigned to those of 4-NH for **4** and 5-NH for **3**, respectively. This observation is consistent with opening of imidazole ring of **Ac**. In case of product **3** two other exchangeable protons observed at δ 9.08 and 8.90 were

assigned to 2-NH and 3-NH respectively. In the spectrum of **4**, a broad signal at δ 7.58 equivalents to four exchangeable protons of two amino groups on sp^3 carbon C-2.

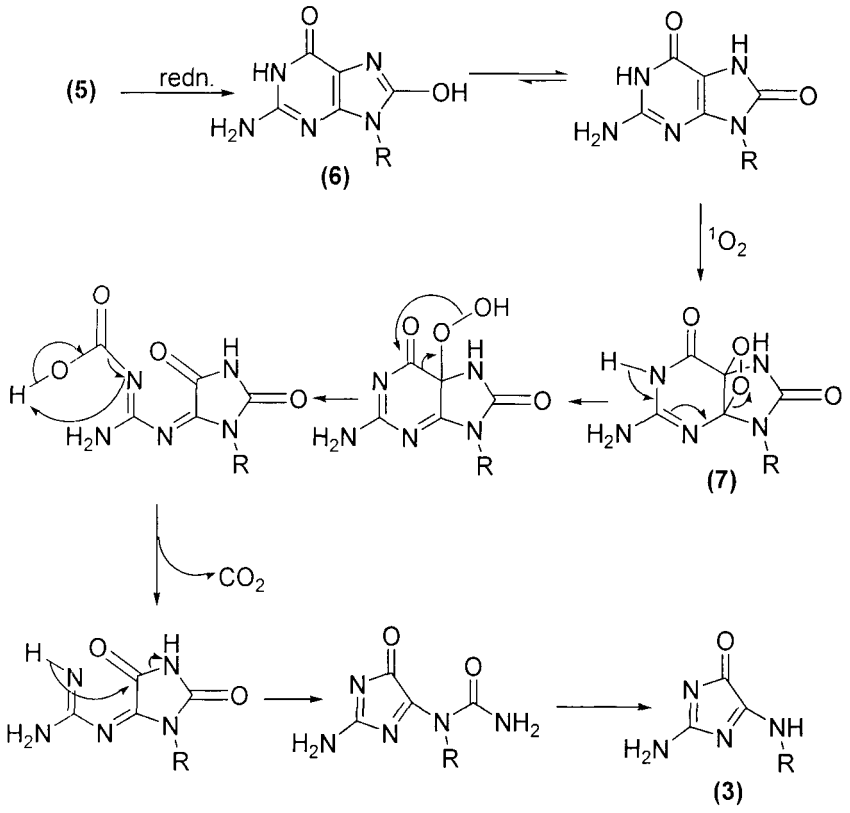
The structure of three products identified in this study is also well supported from their mass spectra. Base peak at m/z 258 corresponds to $[C_8H_{11}N_5O_5+H)]^+$ ion of the oxidation product **2** and peak at m/z 184 corresponds to protonated spiroiminodihydantoin, the expected consequence of fragmentation involving the loss of (hydroxyethoxy)methyl unit. The spectrum of compound **3** exhibits a major peak corresponding to protonated molecule $[C_6H_{10}N_4O_3+H]^+$ at m/z 187 and other peak at m/z 113 $[C_3H_4N_4O+H]^+$ arising from loss of (hydroxyethoxy) methyl unit followed by protonation. The mass spectrum of compound **4** recorded the presence of molecular ion $[C_6H_{12}N_4O_4+H]^+$ at m/z 205 and fragmentation ion $[C_3H_6N_4O_2+H]^+$ at m/z 131, arising from splitting of side chain. The two other fragments $[C_6H_{12}N_4O_4-CO_2+H]^+$ at m/z 161 and $[C_3H_6N_4O_2-CO_2+H]^+$ at m/z 87 may be rationalized by the release of CO_2 from the molecular and fragment ion, respectively. A similar mass fragmentation pattern has already been described for imidazolone-2'-deoxyribonucleoside and oxazolone-2'-deoxyribonucleoside.[15,16] The three products which were identified in this study as, spiroiminodihydantoin **(2)**, imidazolone **(3)** and oxazolone **(4)** are analogous in structure to the products described in photooxidation of deoxyguanosine (dGuo) and its related derivatives.[15-19]

The formation of photoproducts **2**, **3** and **4** has been realized as depicted in scheme 2A.2. The reaction of dienophilic $^1O_2$ with guanine moiety of **Ac (1)** by

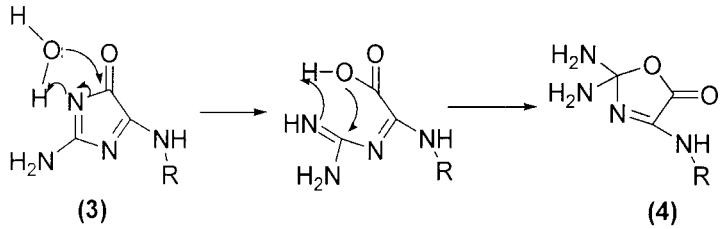
a Diels-Alder [4+2] reaction involving C-4 and C-8 carbons of the purine ring results in the formation of 4,8-purine endoperoxide **(1a)** which isomerizes to 8-hydroperoxyderivative **(5)**. This resulting hydroperoxide may undergo dehydration followed by hydrolytic cleavage to form spiroiminodihydantoin **2** (Scheme 2A.2). Additionally, the hydroperoxide **5** in its reduced form **6** undergoes further [2+2] cycloaddition to produce an unstable dioxetane, which on subsequent decomposition gives imidazole **3** as product (Scheme 2A.3). The sequential formation of imidazolone from unstable dioxetane has its precedence in similar singlet oxygen photooxidation of nucleosides.[20,21] The initially generated photoproduct, namely the imidazolone **(3)**, on hydrolysis leads to the formation of oxazolone derivative **4** (Scheme 2A.4).



Scheme 2A.2



Scheme 2A.3



Scheme 2A.4

[A]. 2. Photooxidation of acyclovir by thermally generated triplet excited ketone from 1,2-dioxetane and its comparison with type I and type II photosensitizers

Biological photosensitization reactions are generally considered as belonging to either the type I (radical mediated) or type II (singlet oxygen mediated).[22] There are many exogenous photosensitizers which impart differential sensitization mechanisms e.g. riboflavin, benzophenone are predominantly type I photosensitizer and rose bengal and methylene blue are type II,[23,24] whereas 3-hydroxymethyl-3,4,4-trimethyl-1,2-dioxetane (HTMD) is neither a typical type I nor a characteristic type II photosensitizer.[25] Hence, in an extensive study, photooxidation of acyclovir was investigated by thermally generated triplet-excited ketone from HTMD for its comparison with type I and type II photosensitizers. The triplet excited ketones are important class of photooxidative sensitizers of biological interest since they may be generated in cellular systems upon exposure of endogenous chromophores to UV radiation or by dark reactions.[26]

Thermal decomposition of HTMD, which is a convenient chemical source of triplet-excited ketone,[25] is accompanied by infrared photoemission at 1270 nm, characteristic for singlet oxygen monomolecular emission,[27] and thus HTMD are known to generate singlet oxygen in sequence, and can operate as type I or type II photooxidants.

The photooxidation of **Ac** was conducted in presence of thermally generated triplet excited ketone. The distribution of the products was compared with those of sensitized photooxidation of **Ac** by riboflavin (type I) and rose bengal (type II).

Experimental

Reaction procedure

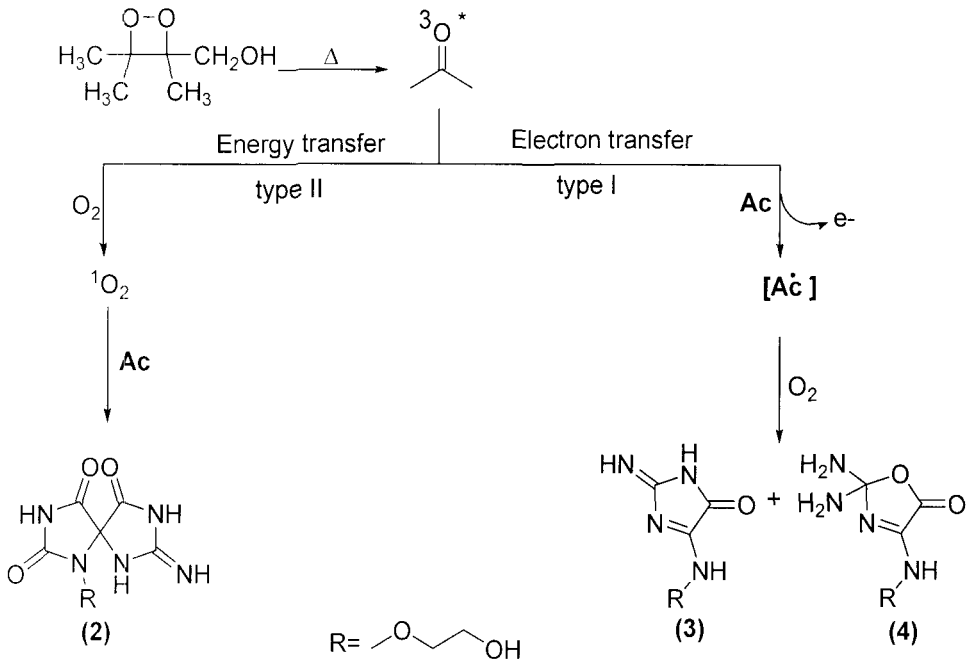
For the HTMD(prepared as per literature[28]) mediated oxidation of **Ac**, a 0.5 mM solution of **Ac** in 10 mM sodium cacodylate buffer (pH 7.0) and 10 vol% of dioxetane solution in acetonitrile was kept at 50° C for 18 h in absence of light. The photosensitized oxidation of phosphate buffered solution of **Ac** (0.5 mM) was carried out in the presence of photosensitizer riboflavin or rose bengal for 2.5 h by irradiation with a 150 W sodium lamp. The lamp was placed at 15 cm below the bottom of an ice filled beaker, in which a round bottom flask having reaction mixture was placed. After the given time period the amount of photoproducts formed and the remaining amount of **Ac** was determined by isolation and purification of the reaction mixture using silica gel column chromatography.

Relative yields of the products was determined on the basis of consumed **Ac** and the mean value of at least 3 independent run, and a comparison of the results with the different sensitizers is presented in Table 2A.1. The concentration and time profile for HTMD induced oxidation of **Ac** (0.5 mM) at 50° C was also determined by using 10 mM sodium cacodylate buffer (pH 7) as a reaction medium with 10 vol% of acetonitrile as co-solvent. Effect of D_2O on the yield of **Sp** in HTMD-induced photooxidation was also observed to confirm the involvement of singlet oxygen.

Results and discussion

On the thermal treatment of **Ac** with **HTMD** at 50° C, three major products were obtained. The products were identified as (2-hydroxyethoxy)methyl spiroiminodihydantoin **(2)**, (2-hydroxyethoxy)methyl (amino)-2-imino-1,2-dihydroimidazole-5-one **(3)**, and 2,2-diamino-4-[(2-hydroxyethoxy)methyl) amino)-5-[2H]-oxazolone **(4)** (Scheme 2A.5). The photoproducts were identified by comparing their spectral data with that of singlet oxygen mediated **Ac** photooxidation products spiroiminodihydantoin **(Sp)** imidazolone **(Im)** and oxazolone **(Ox)**. Similar products pattern was obtained when **Ac** was irradiated in an aerated aqueous solution containing riboflavin or rose bengal, but different yields of products were obtained depending upon the photosensitizer used. (Table 2A.1) In the type II sensitized photooxidation by rose bengal, spiroiminodihydantoin was detected as the major product whereas the type I photosensitizer riboflavin gave imidazolone and oxazolone as major product. For HTMD, which entails both photooxidation modes, the yields of type I and type II products were close to each other. The **Sp/Im+Ox** product ratio indicating the relative predominance of photooxidation products was taken as mechanistic probe to assess the extent of type I and type II photosensitized oxidation. For example in riboflavin sensitized photooxidation the product ratio lies below 1 while for rose bengal sensitized oxidation it is above 3. For HTMD the product ratio is 1.3. Also for the product balance, the HTMD oxidation lies between the type I (40%, entries 2) and type II (57%, entries 3) process.

Furthermore, the relative yield of **Sp** in HTMD-induced oxidation (30%) is significantly higher (15%) than that of riboflavin sensitized oxidation and smaller (15%) than that in the rose bengal sensitization. In contrast the yields of the type I products in HTMD-induced oxidation (23%) are comparable with those of type I sensitizer (25%) and significantly higher than those in type II photooxidation (12%).


Energy transfer
type II
Electron transfer
type I
Ac
e-
[Ac·]
Ac
O2
(2)
(3)
(4)
R= OH

Scheme 2A.5

			Product yields (%)[a]		
Entry	Concentration (μM)	Oxidant	**Sp** (type II)	**Im+Ox** (type I)	Product balance
1	10000	HTMD/ 50°C	30±3	23±2	53±3
2	10	Riboflavin	15±2	25±3	40±3
3	10	Rose bengal	45±1	12±2	57±2

Table 2A.1 Product balance of **Ac** oxidation by dioxetane HTMD and by riboflavin and rose bengal photosensitizers. [a] Relative yields based on consumed **Ac**; mean value of three independent runs.

This difference in products distribution may be accounted for by the fact that rose bengal produce singlet oxygen in large amount by a type II mechanism, whereas riboflavin, which acts mainly by type I photosensitized oxidation, do not produce significant amount of singlet oxygen. On the other hand HTMD is neither a typical type I nor a characteristic type II photooxidant; in particular both photooxidation modes occur quite efficiently.

As shown in Figure 2A.1, when **Ac** was thermally treated with HTMD at 50° C for 15 h, a linearly dependent degradation of **Ac** was observed with increasing HTMD concentration. The absolute yield, based on initial amount of **Ac**, of characteristic type I photooxidation products, **Ox** and **Im** was 15%, while type II product **Sp** was formed in up to 22% absolute yield at 25 mM HTMD concentration. In these reactions the sum of quantified products, relative to consumed **Ac**, amounted to be 56±4 % and was independent of the HTMD concentration. Figure 2A.2 shows the time profile for thermally HTMD induced photooxidation of **Ac**, which revealed a gradual increase of all oxidation products with time and product balance of 53±3 % at 25 mM HTMD. Also in this case the product balance was independent of the reaction time.

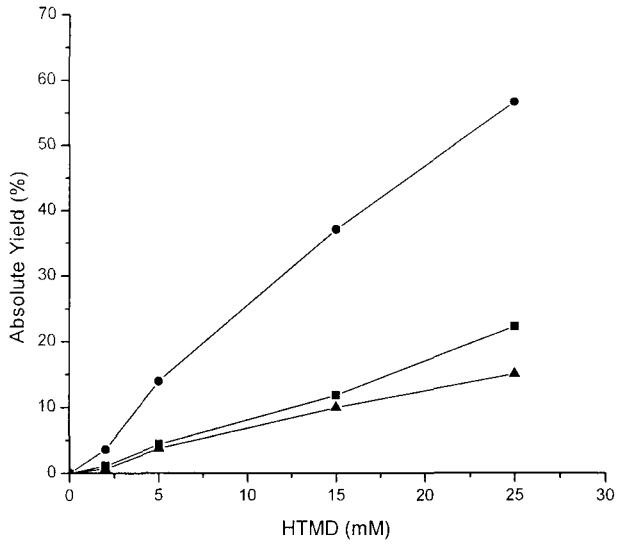


Figure 2A.1 Concentration profile for the thermally HTMD-induced photooxidation of **Ac**, yields derived from the mean values of three independent runs, (●) conversion of **Ac**, (▲) Yields of **Ox** and **Im**, (■) yields of **Sp**.

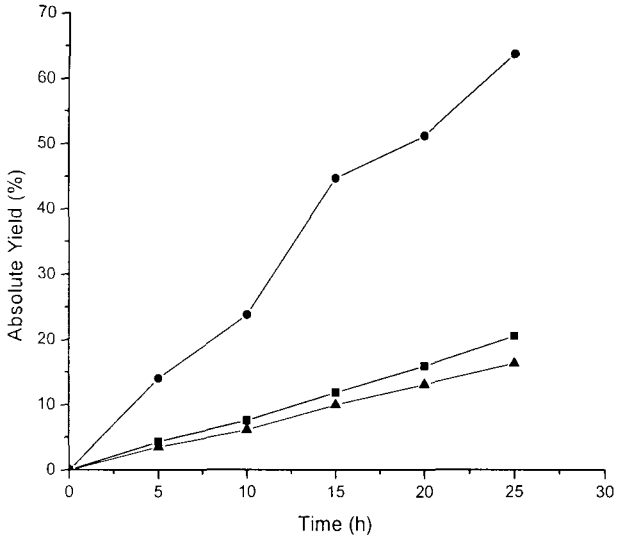


Figure 2A.2 Time profile for the thermally HTMD-induced photooxidation of **Ac**, yields derived from the mean values of three independent runs, (●) conversion of **Ac**, (▲) Yields of **Ox** and **Im**, (■) yields of **Sp**.

The linear increase of **Ac** conversion with increasing HTMD concentration suggested that the HTMD oxidation of **Ac** is directly proportional to the amount of triplet-excited ketone formed by the thermal decomposition of the dioxetane. The time profile for photooxidation of **Ac** exhibited a decrease of **Ac** concentration with time. This reflects that the **Ac** is consumed in parallel with the generation of triplet-excited ketone.

Proof for the involvement of singlet oxygen in HTMD-induced oxidation came from the substantial effect of D_2O on the formation of **Sp**. Higher yields of **Sp** in D_2O compared to those of H_2O indicated that singlet oxygen is involved in HTMD mediated photooxidation. Singlet oxygen quenchers such as DABCO, sodium azide etc. could not be used to confirm the involvement of singlet oxygen in HTMD mediated photooxidation as they are known to react with dioxetane.[29,30]

Our present investigation revealed that triplet excited ketone generated in the thermal decomposition of HTMD oxidizes **Ac** efficiently to the **Sp**, by a type II photooxidation mechanism, and to the **Ox** and **Im** by a type I mechanism. In addition, in the riboflavin sensitized type I oxidative modification of Acyclovir **Ox** and **Im** were obtained as major products, whereas **Sp** was characterized as a major type II photooxidation product of rose bengal sensitized oxidation of **Ac**. The HTMD-induced photooxidation of **Ac** may have an implication to the *in vivo* photobiological transformation in dark and of significance to 'photobiology without light'.[31,32]

Section [B]

Photochemistry of Phenazopyridine Hydrochloride

|B| Photochemistry of Phenazopyridine hydrochloride.

Phenazopyridine hydrochloride (2,6-diamino-3-pheylazopyridine) **(PhPy, 8)** is the generic name for an azo dye, which has been used for 40 years as an analgesic drug to reduce pain, associated with urinary tract infection.[33] Phenazopyridine with an azo chromophore is expected to be photolabile and a probable photosensitizer of biological substrates. Moreover, several drugs with phenylazo moiety are known to biometabolize to arenediazonium ion, which is known to behave as photosensitizer.[34] Interest in the photoreactivity of phenazopyridine arises from the clinical and pharmacological reports of toxic effects[35,36] associated with the use of this drug. 2,3,6-Triaminopyridine **(11)**, a metabolite of phenazopyridine, is known to cause muscle necrosis and renal damage in rats,[37] and it is reasonably anticipated to be human carcinogen,[38] based on sufficient evidence of carcinogenicity in experimental animals.[39,40]

In the present study we have investigated the photolysis of phenazopyridine in different reaction medium, including the drug adsorbed on silica gel, as a biological mimic of situation in liposomes.[41] The results of photolyses are outlined in Scheme 2B.1. The photoreactivity of phenazopyridine was enhanced in the organized medium of silica gel. The various products of photolysis were fully characterized by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometric studies. Formation of 2,3,6-triaminopyridine **(11)** is probably indicative of phototoxicity of drug, as it is identical with the known toxic metabolite of the drug.[37]

Experimental

Apparatus

Same as in section [A]. 1.

Chemicals

All chemical used were of analytical grade. Phenazopyridine hydrochloride **(8)**, was extracted from commercial medicament Pyridium (Parke Davis, India). The purity of drug extracted, was checked by TLC.

Irradiation of phenazopyridine hydrochloride in methanol

Phpy (8) 2 gm (0.009 mole) was dissolved in 100 ml methanol and irradiated in a photoreactor equipped with a 450 W medium pressure mercury vapour lamp inserted in a water cooled quartz fitted immersion well with continuous circulation of water. As the reaction progressed, solution becomes brighter. The progress of the reaction was monitored by TLC using a solvent system of chloroform-methanol (80:20) mixture. After irradiation of the mixture for 12 hours the solvent was removed in a rotary evaporator and crude product was subjected to silica gel column chromatography. Elution with chloroform-petrol gave **9**, **10**, **11** and **12** as products.

Pyrido[3,4-c]cinnoline-2,4-diamine (9): Yield: 35%; UV λ_{max}(MeOH) 252 and 368 nm; HRMS calcd. for (M^+) $C_{11}N_5H_9$ 211.0880, found 211.0860; IR(KBr) : 3500, 3200, 1570, 1200 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.0 (brs, 4H, exch., aromatic C–NH), 5.92 (s, 1H, H-1), 7.57 (m, 3H, H-8, H-9, H-10), 8.30 (d, J=6.5 Hz, 1H, H-7); ^{13}C-NMR ($CDCl_3$) δ 98.3 (C-1), 123.4, 129.6, 131.0, 138.3, 140.5, 146.1, 150 (cinnoline), 151.6 (C-4), 158.1

(C-2); MS m/z (rel. int.) M+1: 212 (100), 214 (12.1), 213 (54.7), 184 (4.9), 183 (5.7) 136 (29.6).

N^3-Phenylpyridine-2,3,4,6-tetraamine (10): Yield: 15%; HRMS calcd. for (M^+) $C_{11}N_5H_{13}$ 215.1218, found 215.1215; IR(KBr) : 3510, 3400, 1590, 1410 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.0 (brs, 7H, exch., aromatic C-NH), 5.20 (s, 1H, H-5), 6.46 (d, J=2.5 Hz, 2H, phenyl), 6.62 (m, 1H, phenyl), 7.01 (m, 2H, phenyl); ^{13}C-NMR ($CDCl_3$) δ 85.4 (C-5), 106.0 (C-3), 116.3, 118.8, 129.6, 143.1 (phenyl), 145.7 (C-4), 148.2 (C-6), 149.2 (C-2); MS: m/z (rel int.) M+1: 216 (7), 215 (45.1), 214 (100.0), 186 (3.9), 185 (6.7), 137 (27.1), 110 (12.7). 109 (9.14), 108 (3.1).

Pyridine-2,3,6-triamine (11): Yield: 12%; HRMS calcd. for (M^+) $C_5N_4H_8$ 124.0904, found 124.0901; IR(KBr): 2925, 1460, 1377, 1320, 763 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.0 (brs, 6H, exch., aromatic C–NH), 5.94 (d, J = 8 Hz, 1H, H-5), 6.64 (d, J = 8 Hz, 1H, H-4); ^{13}C-NMR ($CDCl_3$) δ 100.2 (C-5), 122.0 (C-3), 125.2 (C-4), 147.3 (C-6), 148.3 (C-2); MS: m/z (rel int.) M+1: 125 (70), 108 (63), 81 (30), 54 (100).

2,6-Diamino-1-(4-aminophenyl)pyridin-4(1H)-one (12): Yield: 5%; HRMS calcd. for (M^+) $C_{11}N_4OH_{12}$ 216.1169, found 216.1171; IR(KBr) : 3500, 3430, 1700, 1665 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.0 (brs, 4H, exch., $-NH_2$), 4.0 (brs, 2H, exch., aromatic C-NH), 4.42 (s, 2H, dienone protons), 6.21 (s, 4H, aromatic protons); ^{13}C-NMR ($CDCl_3$) δ 82.6 (C-3 and C-5), 117.1 (C-2', 3', 5', 6'), 131.3 (C-1'), 138.4 (C-4'); MS: m/z (rel. int.) M+1: 217 (31.5), 189 (5.3), 140 (2.3), 139 (28.1), 112 (8.1), 110 (100.0).

Irradiation of Phpy (8) adsorbed on silica gel

The drug was dissolved in methanol and mixed with aqueous slurry of silica gel. TLC plates were prepared and photolyzed as such with a 450 W medium pressure mercury

lamp. The plate appeared as yellow chromatogram, which turned dark yellow within 15 min. Photolysis was continued up to 4 hours for complete decomposition of the drug. The progress of the reaction was monitored by withdrawing a scratch of irradiated silica gel and its co-TLC with the drug. Complete scratch from the plate was dissolved in acetone, filtered and evaporated in a rotary evaporator followed by chromatography on silica gel yielded **9, 10, 11** and **12** as products.

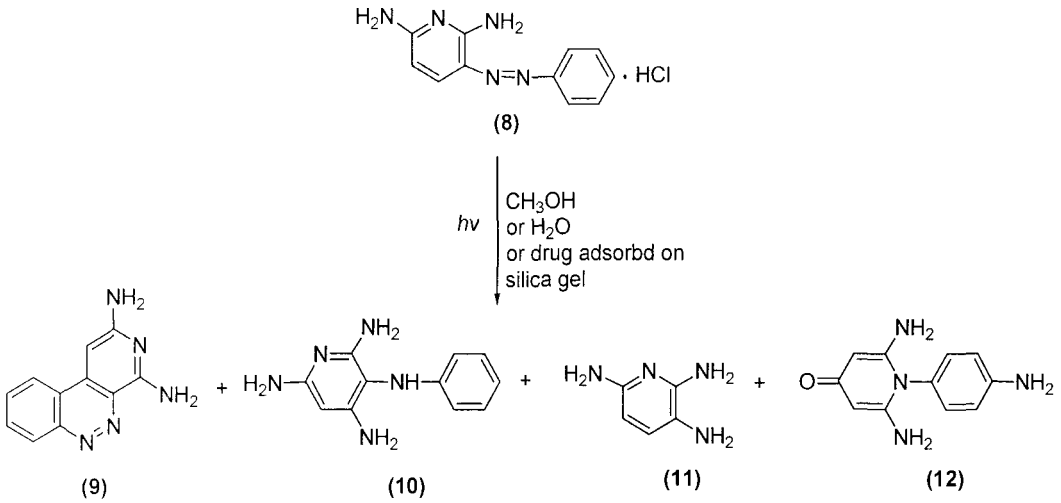
Results and discussion

Irradiation of methanol solution of **Phpy (8)** with medium pressure mercury vapour lamp in a immersion well type photoreactor gave pyrido[3,4-c]cinnoline-2,4-diamine **(9)**, N^3-phenylpyridine-2,3,4,6-tetraamine **(10)**, pyridine-2,3,6-triamine **(11)** and 2,6-diamino-1-(4-aminophenyl)pyridin-4(1H)-one **(12)** as photoproducts (Scheme 2B.1), which were characterized from their spectral studies.

None of these products showed IR band at 1600 cm^{-1} typical for free azo group, however a band at 1570 cm^{-1} in the IR of **9** could be assigned to –N=N- in cinnoline. Photoproduct **9** showed a broad singlet at δ 3.95 ppm equivalent to two $-NH_2$ group protons. A sharp singlet at δ 5.92 ppm was assigned to the only proton present in the diamino substituted pyridine ring. NMR signals for the aromatic ring amounting to only four protons along with characteristic UV bands at 252 and 368 nm supported for a benzo[c]cinnoline[42] structure.

Product **10** showed a broad singlet at δ 4.0 ppm due to protons of aromatic $-NH_2$ group. A sharp singlet at δ 5.2 ppm, logically upfield to the benzene ring protons, was assigned to a single proton flanked by two amino groups in the pyridine ring. This is further supported by the ^{13}C-NMR value of δ 85.4 ppm for the only unsubstituted carbon to

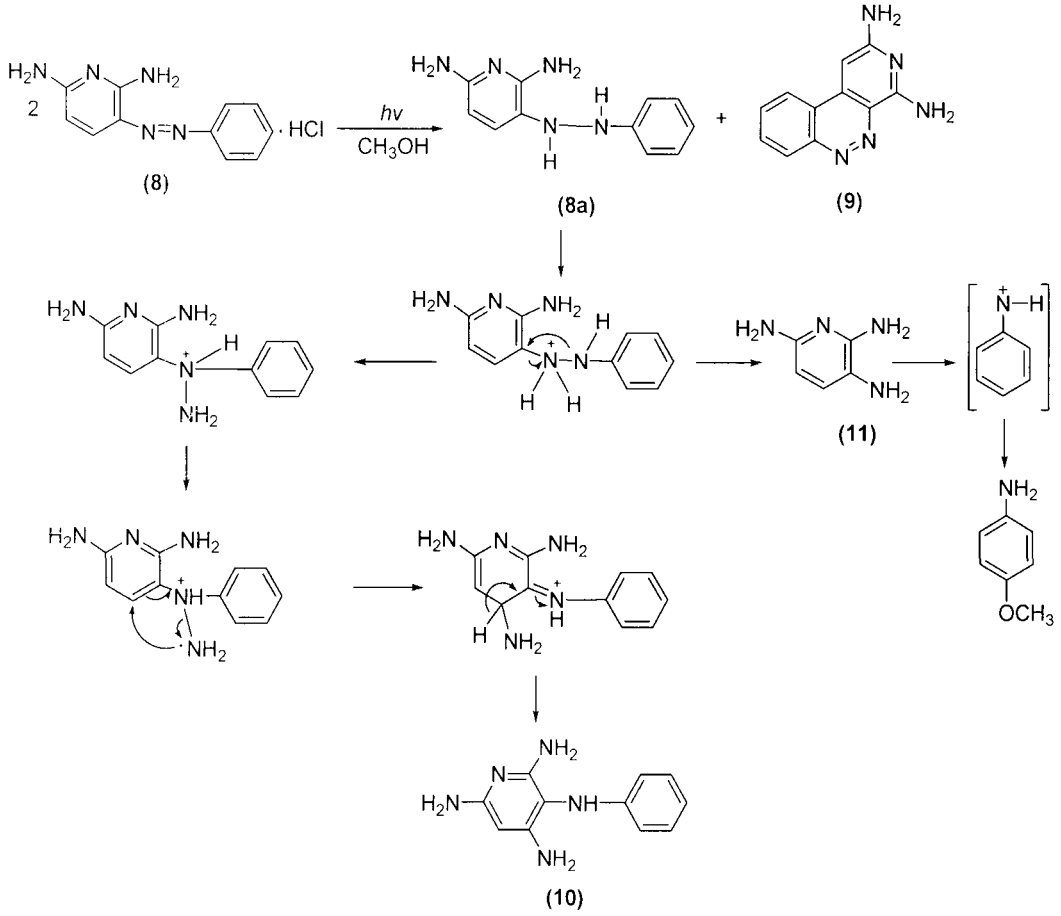
which this hydrogen is attached. The NMR spectrum of product **11**, with a six proton broad singlet at δ 4.0 ppm for aromatic amino group and a pair of ortho coupled doublets at δ 5.94 and 6.64 ppm for aromatic protons, along with its mass spectrum established 2,3,6-triaminopyridine structure for it.



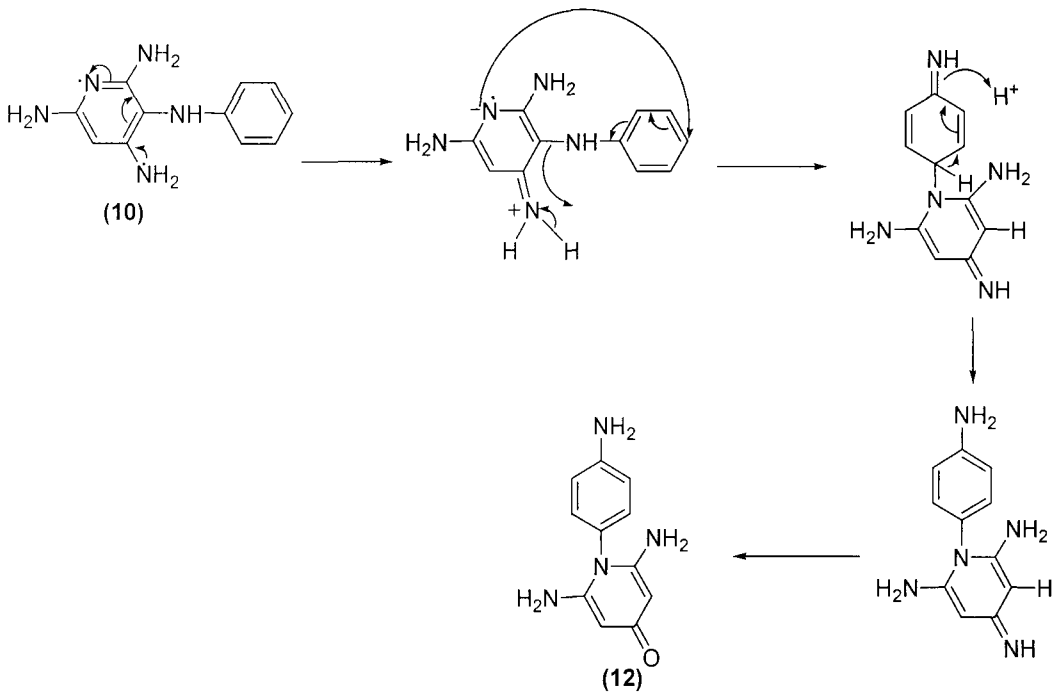
Scheme 2B.1

The photoproduct **12** showed two types of $-NH_2$ signals: at δ 4.0 for two protons and at δ 2.0 for four protons. A sharp singlet for four protons at δ 6.21 ppm indicated that aromatic ring is para-disubstituted. A β,β'-diaminodienone structure for it was supported by proton signal at δ 4.42 ppm for dienone protons and ^{13}C-signal at δ 185.8 ppm for carbonyl carbon and additionally by IR frequency at 1700 and 1665 cm^{-1} (C=O).

The probable course of formation of products is described in scheme 2B.2 and 2B.3. The product **9** results from photochemical cyclodehydrogenation of phenazopyridine, whereby a reduced molecule of the drug **(8a)** also produced.[42] Phenhydrazopyridine **(8a)** undergoes reductive degradation to 2,3,6-triaminopyridine **(11)** and in an alternative course rearranges to N^3-phenylpyridine-2,3,4,6-tetraamine **(10)**. The reductive degradation of **8a** probably co-generates phenylnitrenium ion (Scheme 2B.2), as traces of *p*-methoxyaniline was detected on TLC. Arylnitrenium ions are known intermediates in physiological DNA-damaging reactions, which are responsible for carcinogenesis.[43] 2,6-Diamino-1-4(4-aminophenyl)pyridine-4-(1H)-one **(12)** was proposed to be derived from **10** in a sequence shown in Scheme 2B.3.



Scheme 2B.2


NH2
N
H2N
NH
NH2
(10)
NH2
N
H2N
NH
+N
H
H
NH
H+
H
NH2
N
H2N
H
NH
NH2
NH2
N
H2N
H
NH
NH2
NH2
N
H2N
O
(12)

Scheme 2B.3

References

1. G. M. J. Beijersbergen van Henegouwen, Medicinal photochemistry: Phototoxic and Phototherapeutic Aspects of Drugs, in B. Testa and U. Mayer Ed., Advances in Drug Research Vol. 29, Academic Press, New York and London, 1997, p 79.
2. M. A. Miranda, *Pure Appl. Chem.*, 2001, **73**, 481.
3. G. Cosa, *Pure Appl. Chem.*, 2004, **76**, 263.
4. M. A. Miranda, Phototoxicity of drugs. in *In vitro* methods in pharmaceutical Research. J.V. Castell Ed., Academic press, London, 1997, p 289.
5. B. Quintero, M. A. Miranda, *Ars. Pharmaceutica*, 2000, **41**, 27.
6. http://www.chclibrary.org/micromed/00038150.html
7. W. Haefeli, R. Schoenenberger, P. Weiss, R. Ritz, *Am. J. Med.*, 1993, **94**, 212.
8. S. Feldman, J. Rodman, B. Gregory, *J. Infect. Dis.*, 1988, **157**, 385.
9. De Miranda P, S. Good, *Fed. Proc.*, 1982, **41**, 1733.
10. C. G. Hatchard, C. A. Parker, *Proc. Roy. Soc. (London) A*, 1956, **253**, 518.
11. S. Tamagaki, C. E. Liesner, D. C. Neckers, *J. Org. Chem.*, 1980, **45**, 1573.
12. N. Modric, M. Poje, A. Gojmerac-Ivsic, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1685.
13. F. Johnson, C.-Y.Huang, P.-L. Yu, *Environ. Health. Perspect.*, 1994, **102**, 143.
14. M. Poje, E. F. Paulus, B. Rocic, *J. Org. Chem.*, 1980, **45**, 65.

15. G. R. Martinez, M. H. G. Medeiros, J.-L. Ravanat, J. Cadet, P. D. Mascio, *Biol. Chem.*, 2002, **383**, 607.

16. J.-L. Ravanat, G. Remaud, J. Cadet, *Arch. Biochem. Biophys.*, 2000, **374**, 118.

17. J. Cadet, M. Berger, T. Douki, J.-L. Ravanat, *Rev. Physiol. Biochem. Pharmacol.*, 1997, **131**, 1.

18. J.-L. Ravanat, J. Cadet, *Chem. Res. Toxicol.*, 1995, **8**, 379.

19. W. Adam, M. A. Arnold, M. Grune, W. M. Nan, U. Pischel, C. R. Saha-Moller, *Org. Lett.*, 2002, **4**, 537.

20. G. W. Buchko, J. R. Wagner, J. Cadet, S. Raoul, M. Weinfeld, *Biochim. Biophys. Acta.*, 1995, **1263**, 17.

21. S. Raoul, J. Cadet, *J. Am. Chem. Soc.*, 1996, **118**, 1892.

22. C. S. Foote, *Photochem. Photobiol.*, 1991, **54**, 659.

23. J. Cadet, C. Deccaroz, S. Y. Wang, W. R. Midden, *Isr. J. Chem.*, 1983, **23**, 420.

24. G. W. Buchko, J. Cadet, *Can. J. Chem.*, 1992, **70**, 1827.

25. W. Adam, G. Cilento, *Angew. Chem. Int. Ed. Engl.*, 1983, **22**, 529.

26. B. Epe, H. Henzl, W. Adam, C. R. Saha-moller, *Nucleic Acids Res.*, 1993, **21**, 863.

27. K. Briviba, C. R. Saha-Moller, W. Adam, H. Sies, *Biochem. Mol. Biol., Int.* 1996, **38**, 647.

28. D. Leclercq, J. -P. Bats, P. Picard, J. Moulines, *Synthesis* 1982, 778.

29. A. R. Sundquist, M. Hanusch, W. Stahl, H. Sies, *Photochem. Photobiol.*, 1993, **57**, 785.

30. W. Adam, M. Heil, *J. Am. Chem. Soc.*, 1992, **114**, 5591.

31. B. Epe, E. Muller, W. Adam, C. R. Saha-Moller, *Chem. Biol. Interact.*, 1992, **85**, 265.

32. W. Adam, M. Ahrweiler, C. R. Saha-Moller, M. Sauter, A. Schonberger, B. Epe, E. Muller, D. Schiffmann, H. Stopper, D. Wild, *Toxicol. Lett.*, 1993, **67**, 41.

33. F. Doerge., anti-infective agents, Textbook of Organic Medicinal and Pharmaceutical Chemistry, in C. O. Wilson, O. Gisvold and R. F. Doerge Ed., J. B. Lippincott company, Toronto, 7th ed., 1977, p 160.

34. B. Quintro, M. A. Miranda, *Ars Pharmaceutica*, 2000, **41**, 27.

35. H. Narchi, S. Aramo, *Pediatr. Rev.*, 2000, **21**, 91.

36. R. Munday, E. A. Fowke, *Free radical Res.*, 1994, **21**, 67.

37. R. Munday, E. Manns, *J. Appl. Toxicol.*, 1998, **18**, 161.

38. IARC 1987, IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, Suppl. 7, Lyon, France, International agency for research on cancer, p 440.

39. IARC 1980, IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, vol 24, Lyon, France, International agency for research on cancer, p 337.

40. IARC 1982, IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, Suppl. 4, Lyon, France, International agency for research on cancer, p 292.

41. J. K. Yamamoto, R. F. Borch, *Biochemistry* 1985, **24**, 3338.

42. G. M. Badger, R. J. Drewer, G. E. Lewis, *Aust. J. Chem.*, 1963, **16**, 1042.

43. R. J. Robbins, D. M. Laman, D. E. Falvey, *J. Am. Chem. Soc.*, 1996, **118**, 8127.

Chapter 3

Photochemistry of Bischromophoric Steroidal Anti-inflammatory Drugs (Desonide and Clobetasol propionate)

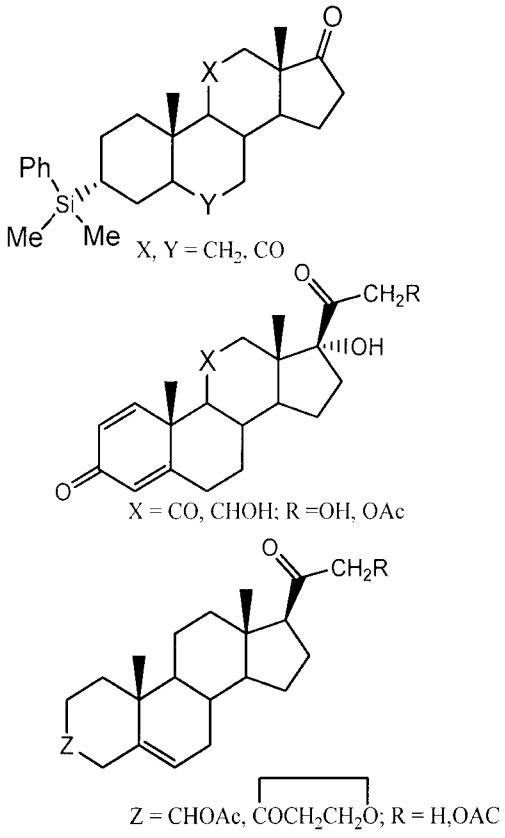
Introduction

Polyfunctional molecules in which different photochemically reactive chromophores are connected by rigid hydrocarbon framework are a subject of fascinating photochemistry.[1-3] The intramolecular energy transfer (both singlet-singlet and triplet-triplet) may occur from an 'antenna' group to other chromophore leading to chemistry different from that observed by direct excitation of that chromophore.[4-7] In the photochemistry of such multichromophoric molecules the evaluation of interaction between the chromophores, the mode and extent of local reaction at any chromophore after electronic excitation and possible role of energy transfer is of high mechanistic significance.

Morrison established, through a series of elegant papers,[4-7] that intramolecular energy transfer (both singlet-singlet and triplet-triplet) occurred from the phenyl 'antenna' to C_{17} keto group in the steroids (Scheme 3.1) by the way of through-bond mechanism. This lead to a different photochemistry observed by the direct excitation of ketone chromophore. Albini et al.[8,9] have demonstrated non-communicating reaction paths in some pregna-1,4-diene-3,20-dione. Since many steroidal drugs are commonly used and several reports on their phototoxic effects have been reported,[10-13] it was of interest to extensively study the aspect of competition between chemical reactions of the separated excited moieties incorporated in the rigid skeleton of the steroids. It was expected that such photochemical mechanisms might have some relevance for the mechanism of phototoxicity.

Glucocorticosteroids are natural hormones with a steroidal structure derived from 5α-pregnane. These steroidal hormones with powerful anti-inflammatory effects are secreted by the cortex of adrenal gland. Semisynthetic derivatives of these hormones are widely used as drugs to treat inflammatory illness, including arthritis and asthma, and many of them are effective by topical use in dermatoses and other dermatological disease.

With this interest, herein we have investigated the photochemistry of desonide **(De, 1)** and clobetasol propionate **(6)** under different combinations of solvents and irradiation wavelengths.



Scheme 3.1

[A] Photochemical Studies on desonide

Desonide **(De, 1)** is a synthetic nonfluorinated corticosteroid for topical dermatological use. It is used to treat inflammation caused by a number of conditions such as allergic reactions, asthma and psoriasis.[14,15] Desonide is very interesting from photochemical point of view because it bear two spatially separated chromophores i.e. cross conjugated dienone moiety in ring A and an isolated ketone at C_{20}. A number of photochemical studies have been carried out on steroidal ketones, both in solution and in solid state.[16-22] The photochemistry of cross-conjugated cyclohexadienone has been intensively studied because of their facile and fascinatingly complex photochemical reactions. William et al.[23] carried out photolysis of prednisolone, a molecule structurally related to **De**. at 254 nm and observed that only photoprocess occurring in dioxane solution was the "lumiketone" rearrangement of the cyclohexadienone moiety.

Experimental

Chemicals and Instrumentation

All chemicals used were of analytical grade and were used as such without any further purification. Pure desonide and clobetasol propionate were obtained from Galderma Pvt. Ltd. (Mumbai, India) and ZYG Pharma (Mumbai, India) respectively. Irradiation at 254 nm were carried out in an immersion well type photoreactor (quartz) equipped with 20 W low-pressure mercury arc lamp. For irradiations at 310 nm the solutions were irradiated with two 15 W phosphor coated lamps. IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RX1. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 spectrometer using $SiMe_4$ as internal standard and $CDCl_3$ as

solvent. Circular dichroism spectra were measured on a Jasco-J 41A spectropolarimeter. High-resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 eV ionization voltage. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70-230 mesh).

General photoirradiation procedure

A solution of desonide or clobetasol (in acetonitrile or 2-propanol) was stirred and flushed with argon or oxygen (as desired) for 1 h before irradiation and was kept bubbling during the irradiations. The course of reaction was monitored by thin layer chromatography on pre-coated silica gel TLC plates using chloroform-acetone (9:1) mixture. After the completion of reaction (when desired conversions have reached) the solvent was removed in a rotary evaporator and products were purified by silica gel column chromatography.

Irradiation of De in argon- saturated acetonitrile

A solution of **De** (210 mg, 0.5 mM) in argon-saturated acetonitrile (400 ml) was irradiated for 2.5 h at 254 nm. After following the steps described in general photoirradiation procedure, compound **2** was obtained as product.

11β,21-Dihydroxy-16α,17α-(1-methylethylidenedioxy)-1,5-cyclopregn-3-ene-2,20-dione (2): Yield: 115 mg (55%); HRMS calcd. for (M^+) $C_{24}H_{32}O_6$ 416.5073, found 416.5065; IR (KBr) 3410,1680 (C=O), 1565 (C=C), 1355, 1160, 1022 cm^{-1} (cyclopropyl); ^{1}H-NMR (DMSO-d_6) δ 7.24 (d,1H, J=6Hz, H-4), 5.98 (d, 1H, J=6Hz, H-3), 4.87 (brs, 1H, exch., OH), 4.69 (s, 2H, H-21), 4.10 (t, 1H, H-16), 3.98 (brs, exch., OH), 3.16 (dd, 1H, J=11,5 Hz, H-11), 1.82 ($OC(\underline{C}H_3)_2$, 6H), 1.6-1.8 (m, 5H), 1.2-1.5

(m, 6H), 1.16 (s, 3H, H-19), 1.08 (s, 1H, H-1), 0.95 (s, 3H, H-18); ^{13}C-NMR (DMSO)-d_6) δ 211.4 (C-20), 193.2 (C-2), 132.5 (C-3), 158.1 (C-4), 109.2 (O<u>C</u>$(CH_3)_2$), 98.7 (C-17), 81.4 (C-16), 65.2 (C-11), 64.6 (C-21), 58.2 (C-9), 47.4 (C-1), 48.2 (C-14), 40.4 (C-12), 36.6 (C-13), 35.1 (C-6), 32.1 (C-5), 29.9 (C-10), 27.4 (C-8), 26.0 (C-15), 25.3 (C-7), 20.8 (C-19), 14.4 (C-18).

A solution of **De** (210 mg, 0.5 mM) in argon-saturated acetonitrile (400 ml) was irradiated for 2 h at 310 nm. After following the steps described in general photoirradiation procedure, compound **3** was obtained as the main product along with a trace amount of **2** as detected on TLC.

11β-Hydroxy-16α,17α-(1-methylethylidenedioxy) androsta-1,4-diene-3-one (3): yield: 105 mg (50%); HRMS calcd. for (M^+) $C_{22}H_{30}O_4$ 358.4712, found 358.4718; IR (KBr) 3500,1670, 1625, 1610; ^{1}H-NMR (DMSO-d_6) δ 7.52 (d, J=8 Hz, 1H, H-1), 6.14 (d, 1H, J=8 Hz, H-2), 6.03 (s, 1H, H-4), 3.92 (m, 1H, H-16), 3.87 (d, J-5Hz, 1H, H-17), 3.40 (brs.1H. exch., OH), 3.26 (m, 1H, H-11), 1.6-2.0 (m, 6H), 1.4-1.6 (m, 5H), 1.37 (s, 3H, H-19), 1.31 (s, 6H), 1.16 (s, 3H, H-18); ^{13}C-NMR (DMSO-d_6) δ 185.8 (C-3), 168.3 (C-5), 155.4 (C-1), 128.4 (C-2), 124.2 (C-4), 113.3 (O<u>C</u>$(CH_3)_2$), 80.4 C-16), 103.3 (C-17), 66.7 (C-11), 59.0 (C-9), 46.7 (C-14), 43.1 (C-12), 34.4 (C-10), 33.6 (C-6), 32.0 (C-7), 31.6 (C-13), 29.9 (C-8), 28.7 (C-15), 26.6 (OC(<u>C</u>$H_3)_2$), 25.7 (C-19), 17.3 (C-18).

Irradiation of De in oxygen saturated acetonitrile

A solution of **De** (210 mg, 0.5 mM) in oxygen-saturated acetonitrile (400 ml) was irradiated for 2.5 h at 254 nm and 310 nm. After following the steps described in

general photoirradiation procedure, compound **2** (140 mg, 67%) was obtained as product at 254 nm whereas a complex mixtures of products was obtained at 310 nm.

Irradiation of De in argon-saturated 2-propanol

A solution of **De** (210 mg, 0.5 mM) in argon-saturated 2-propanol (400 ml) was irradiated for 2 h at 254 nm and at 310 nm. After following the steps described in general photoirradiation procedure, compound **2** (130 mg, 62%) was obtained as major product at 254 nm. Whereas at 310 nm both the compounds **2** and **3** were obtained as products.

Irradiation of De in oxygen-saturated 2-propanol

A solution of **De** (210 mg, 0.5 mM) in acetonitrile (400 ml) was irradiated for 2.5 h at 254 nm and 310 nm. After following the steps described in general photoirradiation procedure compound **2** (135 mg, 64%) was obtained as product at 254 nm. At 310 nm **2** (55 mg, 26%) and **4** (110 mg, 52%) were obtained as products.

17β-Hydroperoxy-11β- hydroxy-16α, 17α-(1-methylethylidenedioxy) androsta-1,4-diene-3-one (4): Yield: 110 mg (52%); HRMS calcd. for (M^+) $C_{22}H_{30}O_6$ 390.4700, found 390.4694; IR (KBr) 3400, 1655, 1620, 1600; ^{1}H-NMR (DMSO-d_6) δ 8.9 (brs, exch., OOH), 7.56 (d, J=8 Hz, 1H, H-1), 6.18 (d, 1H, J=8 Hz, H-2), 6.08 (s, 1H, H-4), 4.12 (m, 1H, H-16), 4.82 (brs, 1H, exch., OH), 2.2-2.6 (m, 4H), 1.2-1.9 (m, 6H), 1.4 (s, 3H), 1.3 (s, 3H), 1.2 (s,3H); ^{13}C-NMR (DMSO-d_6) δ 192.0 (C-3), 167.1 (C-5), 156.4 (C-1), 125.7 (C-2), 124.4 (C-4), 121.5 (C-17), 105.4 ($O\underline{C}(CH_3)_2$), 73.8 (C-16), 67.1 (C-11), 58.6 (C-9), 40.8 (C-14), 37.1 (C-10), 36.2 (C-12), 33.1 (C-6), 31.9 (C-7), 30.4 (C-13), 29.6 (C-8), 22.8 (C-15), 26.4 ($OC(\underline{C}H_3)_2$), 25.4 (C-19), 17.5 (C-18).

Triphenylphosphine (26 mg) was added to a solution of hydroperoxide **4** (20 mg) in dichloromethane (20 ml) and stirring was pursued for 2 h, when the starting material was consumed, extraction with water and evaporation gave 15 mg of Compound **5**.

11β,16α-Dihydroxyandrosta-1,4-diene-3,17-dione (5): HRMS calcd. for (M^+) $C_{19}H_{24}O_4$ 316.3915, found 316.3920; IR (KBr) 3450, 1745, 1660, 1600; ^{1}H-NMR (DMSO-d_6) δ 7.31 (d, J=8 Hz, 1H, H-1), 6.42 (d, 1H, J=8 Hz, H-2), 6.01 (s, J=1.5Hz, 1H, H-4), 4.98 (brs, 1H, exch., OH), 4.10 (m, 1H, H-16), 4.01 (brs, 1H, exch., OH), 2.5-2.8 (m, 3H), 1.7-2.4 (m, 5H), 1.3-1.7 (m, 4H), 1.41 (s, 3H), 0.98 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ 205.4 (C-17), 181.3 (C-3), 165.2 (C-5), 152.5 (C-1), 130.1 (C-2), 127.2 (C-4), 75.2 (C-16), 67.4 (C-11), 58.3 (C-9), 40.8 (C-10), 40.4 (C-19), 39.7 (C-13), 36.7 (C-14), 32.0 (C-6), 31.5 (C-8), 30.5 (C-12), 29.6 (C-7), 27.7 (C-15), 21.2 (C-18).

Results and discussion

Irradiation of **De** at 254 nm in argon flushed acetonitrile or in oxygen-saturated solution gave compound **2** as product. The photoreaction of **De** in 2-propanol at 254 nm followed a similar course of reaction under aerobic as well as anaerobic conditions. When argon flushed **De** solution (acetonitrile or 2-propanol) was irradiated at 310 nm two products were obtained in both the solvents, which were identified as **2** and **3**. At the same irradiation wavelength (310 nm) saturation of the solution with oxygen affected the product distribution: in 2-propanol **3** was not formed, instead hydroperoxide **4** was obtained as main product along with trace amount of **2**. Whereas in acetonitrile a complex mixture of products was obtained (Scheme 3.2).

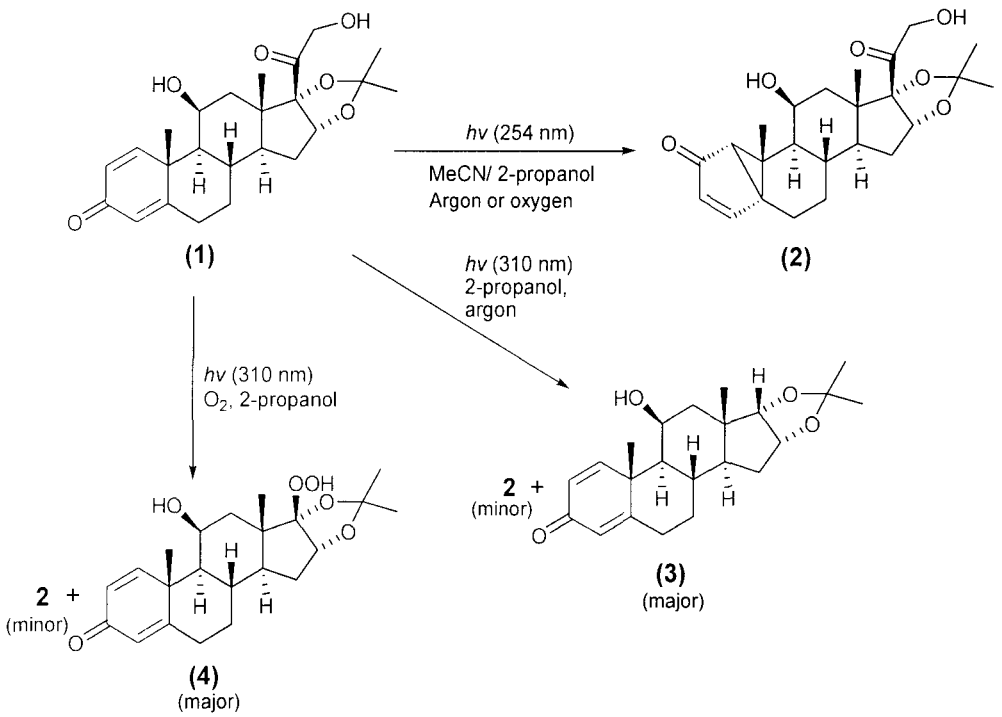
These results can be rationalized on the basis of different mechanism of photochemical reaction of the two-separated chromophores present in this drug. At 254 nm, cross-conjugated ketone absorbs predominantly or exclusively, which causes the well known lumiketone rearrangement[24,25] of this chromophore and leads to the formation of compound **2** (Scheme 3.3). The rearrangement leading to **2** is a concerted process and therefore not affected by the medium. On the contrary at 310 nm, where isolated ketone at C_{20} absorbs a large fraction of light, compound **3** was obtained as product which arises via Norrish Type I homolytic photocleavage of C_{17}-C_{20} bond followed by hydrogen atom abstraction by alkyl radical from solvent or from $HOCH_2CO$ radical. In oxygen saturated solution trapping of alkyl radical by oxygen is quite efficient to yield peroxy radical. This peroxy radical abstracts hydrogen from protic solvent (2-propanol) to give the isolated hydroperoxy derivative **4** (Scheme 3.4).

All the products obtained were characterized on the basis of the following spectral evidences. The IR spectrum of **2** showed absorption bands at 1355, 1160, 1022 (cyclopropyl), 1565 (C=C), 1680 (C=O). In the NMR spectrum of **2**, signals due to the rings B, C and D were found to be unaffected while signals due to ring A was strongly modified since only two of the olefinic CH were conserved and third was substituted by a sp^3 carbon. In addition two doublets centered at δ 7.24 and 5.98 with J=6.0 Hz in the ^{1}H-NMR spectrum and the IR band values indicated the presence of an α,β-unsaturated ketone in ring A. A proton singlet at δ 1.08 and ^{13}C-NMR signals at 47.4, 32.1 and 29.9 indicated a cyclopropyl carbonyl system in ring A. Proof of the stereochemistry came from a comparison of its circular dichroism spectra with those of other lumiketones that

showed positive and negative cotton effects of similar magnitude and position to those reported in litrature[26,27] (Table 3.1).

Spectroscopic study of compound **3**, particularly NMR data indicated that the steroidal skeleton was unaffected while the side chain at C_{17} has been lost. In the ^{1}H-NMR spectrum of **3** the three deshielded olefinic protons at δ 7.52 (d, J=8 Hz, 1H), 6.14 (d, J=8 Hz, 1H) and 6.03 (s, 1H) confirmed that the dienone system was intact, and on the basis of chemical shifts and spin-spin coupling constants these signals were assigned to the C-1, C-2 and C-4 protons respectively. Signals due to ring B, C and D were also unaffected while no signal was observed due to side chain at C_{17} in its NMR spectrum. Its IR spectrum with absorption bands at 3500 (OH), 1670 (α,β-C=O), 1625, 1610 (C=C) cm^{-1}, further support the assigned structure **3** for this compound.

The NMR spectra of **4** suggested that the structural features in ring A, B and C were again conserved while side chain at C_{17} had been lost. In addition a strongly deshielded signal at δ 8.9 (brs, exch., 1H) in the ^{1}H-NMR and a new signal at δ 121.5 (C-17) in ^{13}C-NMR suggested the presence of hydroperoxy group in **4**. This compound could be reduced by triphenylphosphine and gave 16-hydroxy-17-keto derivative **5** as product (Scheme 3.5). This chemical evidence along with the spectroscopic indications (in the experimental section) allowed assignment of the hydroperoxide structure **4** for the product.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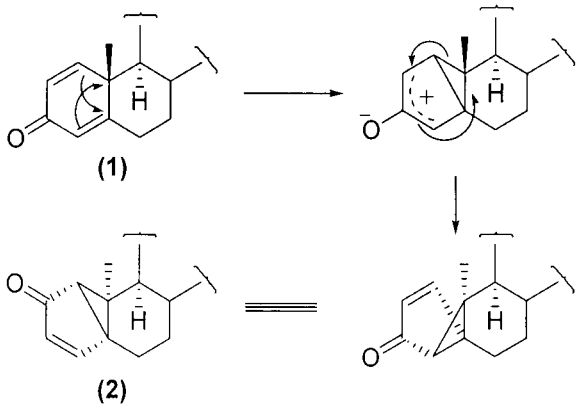
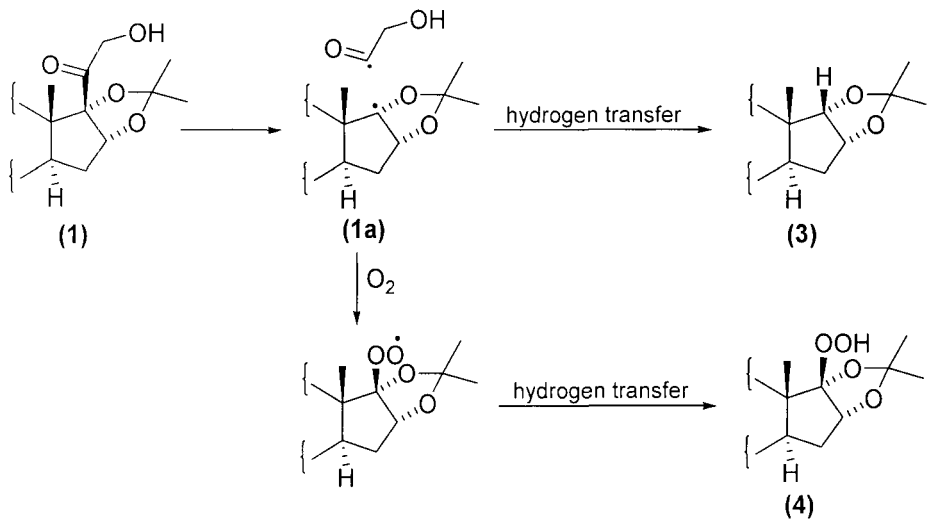
O
(1)
hv (254 nm)
MeCN/ 2-propanol
Argon or oxygen
(2)
hv (310 nm)
2-propanol,
argon
hv (310 nm)
O2, 2-propanol
OOH
2 +
(minor)
(4)
(major)
(3)
(major)

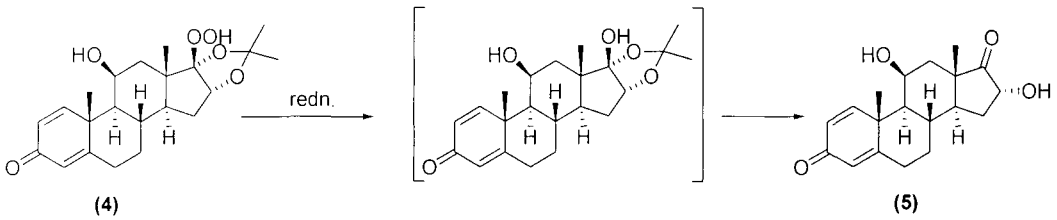
Scheme 3.2



Scheme 3.3


OH
O
O
O
H
(1)
OH
O
O
O
H
(1a)
hydrogen transfer
H
O
O
H
(3)
O2
OO
O
O
H
hydrogen transfer
OOH
O
O
H
(4)

Scheme 3.4



Scheme 3.5

Compd.	λ_{max} ($\Delta\varepsilon$)	λ_{max} ($\Delta\varepsilon$)	Crossover λ	λ_{max} ($\Delta\varepsilon$)	Crossover λ	λ_{max} ($\Delta\varepsilon$)
2		343 (-4.71)	313	278 (+12.22)	253	225 (-11.71)
Related lumiproducts[a]	357 (-3.71)	344.5 (-3.77)	309	272 (+10.3)	250	Short wavelength –ve CD

[a]see refs. [26, 27]

Table 3.1 Circular dichroism spectra of **2** and other related lumiproducts

[B] Photochemical studies on clobetasol propionate

Clobetasol propionate **(6)** is a dihalogenated highly potent glucocorticoid. It is an analogue of prednisolone. It is used only topically on the skin and its effects are limited to the local anti-inflammatory activity. Clobetasol is indicated for the treatment of psoriasis,[28] dry hyperkeratotic dermatoses, initial control of all forms of hyperacute eczema, chronic hyperkeratotic eczema, contact dermatitis, atopic dermatitis, lichen planus associated with severe pruritis, discoid lumps erythema and granulomatous disorders.[29,30]

Clobetasol is also very interesting from photochemical point of view because it also bears two spatially separated chromophores i.e. cyclohexadienone moiety in ring A and carbonyl group at C_{20}. Hence, in an extensive study of photochemistry of steroidal anti-inflammatory drugs, we have investigated the photochemistry of clobetasol under different combinations of solvents and irradiation wavelengths.

Experimental

Same as in section [A]

Irradiation of clobetasol in argon- saturated acetonitrile

A solution of **6** (234 mg, 0.5 mM) in argon-saturated acetonitrile (400 ml) was irradiated for 2.5 h at 254 nm. After following the steps described in general photoirradiation procedure (section A), 7 (110 mg) was obtained as product.

21-Chloro-9-fluoro-11-hydroxy-16-methyl-17(1-oxopropoxy)-1,5-cyclopregn-3-ene-2,20-dione (7): Yield: 110 mg (47%); HRMS calcd. for (M^+) $C_{25}H_{32}O_5ClF$ 466.9771, found 466.9780; IR (KBr) 3400, 1675 (C=O), 1570 (C=C), 1350, 1161, 1030 cm^{-1}

(cyclopropyl); ^{1}H-NMR (DMSO)-d_6) δ 6.66 (d,1H, J=6.2 Hz, H-4), 5.68 (d, 1H, J=6.2 Hz, H-3), 4.42 (s, 2H, H-21), 4.32 (brs, exch., OH), 2.53 (m, 1H, H-16), 3.43 (dd, 1H, J=11.5 Hz, H-11), 2.29 (m, 2H, H-23), 1.74 (m, 1H, H-8), 1.5-1.7 (m, 6H), 1.42 (dd, 1H, J=11, 2 Hz, H-14), 1.32 (m, 2H, H-15), 1.21 (6H, 2×CH_3), 1.11 (6H, 2×CH_3) 1.06 (s, 1H, H-1); ^{13}C-NMR (DMSO)-d_6) δ 201.3 (C-20), 192.5 (C-2), 174.4 (C-22), 159.7 (C-4), 130.8 (C-3), 114.1 (C-9), 93.9 (C-17), 71.1 (C-11), 41.3 (C-21), 40.4 (C-1), 39.3 (C-14), 38.1 (C-8), 37.4 (C-6), 37.0 (C-13), 32.9 (C-12), 31.8 (C-15), 31.2 (C-16), 27.8 (C-23), 27.1 (C-10), 25.2 (C-5), 18.7 (C-7), 14.6 (C-18) 9.4 (C-19).

A solution of **6** (234 mg, 0.5 mM) in argon-saturated acetonitrile (400 ml) was irradiated for 2 h at 310 nm. After following the steps described in general photoirradiation procedure (section A), **8** (90 mg) was obtained as main product along with trace amount of **7**, as detected on TLC.

21-Chloro-9-fluoro-11-hydroxy-16-methyl-17(1-oxopropoxy)-18,20-cyclopregn-1,4-diene-3-one (8): Yield: 90 mg (39%); HRMS calcd. for (M^+) $C_{25}H_{32}O_5ClF$ 466.9771, found 466.9765; IR (KBr) 3410,1680, 1630, 1615; ^{1}H-NMR (DMSO)-d_6) δ 7.34 (d, J=9 Hz, 1H, H-2), 6.28 (d, J=9 Hz, 1H, H-1), 6.09 (s, 1H, H-4), 3.77 (s, 2H, H-21), 3.51 (brs, exch., OH), 3.34 (m, 1H, H-11), 2.37 (m, 1H, H-16), 2.21 (m, 2H, H-22), 2.11 (s, 2H, H-18), 2.09 (d, 2H, H-6), 1.74 (m, 1H, H-8), 1.59 (m, 2H, H-15), 1.52 (m, 2H, H-7), 1.36 (3H, CH_3), 1.14 (3H, CH_3), 1.06 (3H, CH_3); ^{13}C-NMR (DMSO)-d_6) δ 185.8 (C-3), 173.4 (COOR), 167.2 (C-5), 154.7 (C-1), 129.2 (C-2), 125.4 (C-4), 102.2 (C-17), 99.8 (C-9), 98.1 (C-20), 72.2 (C-11), 55.3 (C-10), 46.9 (C-21), 42.1 (C-14), 39.4 (C-8),

37.0 (C-13), 35.2 (C-12), 34.9 (C-18), 33.6 (C-6), 28.7 (C-16), 25.8 (C-7), 19.2 (CH_3), 17.4 (CH_3), 16.1 (CH_3).

Irradiation of clobetasol in oxygen saturated acetonitrile

A solution of **6** (234 mg, 0.5 mM) in oxygen-saturated acetonitrile (400 ml) was irradiated for 2.5 h at 254 nm and 310 nm. After following the steps described in general photoirradiation procedure (section A), compound **7** (112 mg, 48%) was obtained as product at 254 nm where as a complex mixtures of products was obtained at 310 nm.

Irradiation of clobetasol in argon-saturated 2-propanol

A solution of **6** (234 mg, 0.5 mM) in argon-saturated 2-propanol (400 ml) was irradiated for 2 h at 254 nm and at 310 nm. After following the steps described in general photoirradiation procedure (section A), compound **7** (120 mg, 52%) was obtained as major product at 254 nm. Whereas at 310 nm both the compounds **7** and **8** were obtained as products.

Irradiation of clobetasol in oxygen-saturated 2-propanol

A solution of **6** (234 mg, 0.5 mM) in oxygen-saturated acetonitrile (400 ml) was irradiated for 2.5 h at 254 nm and 310 nm. After following the steps described in general photoirradiation procedure (section A), compound **7** (130 mg, 56%) was obtained as product at 254 nm. At 310 nm **7** (42 mg, 18%) and **9** (100 mg, 43%) were obtained as products.

9-Fluoro-17-hydroperoxy-16-methyl-17(1-oxopropoxy) androsta-1,4-diene-3-one (9):

Yield: 100 mg (43%); HRMS calcd. for (M^+) $C_{23}H_{31}O_6F$ 422.6939, found 422.6930; IR

(KBr) 3410, 1660, 1615, 1610; ^{1}H-NMR (DMSO)-d_6) δ 8.7 (brs, exch., OOH), 7.14 (d, 1H, J=9 Hz, H-2), 6.30 (d, J=9 Hz, 1H, H-1), 6.09 (s, 1H, H-4), 3.43 (m, 1H, H-11), 2.29 ((m, 2H, -OOCCH_2CH_3), 2.35 (m, 1H, H-16), 2.1 (brs, exch., OH), 1.5-1.7 (m, 5H, H-8, H-12, H-15), 1.36 (s, 3H, H-19), 1.16 (s, 3H, H-18), 2.0 (m, 2H, H-6), 1.52 (m, 2H, H-7), 1.40 (s, 1H, H-14), 1.11 (3H, CH_3), 1.02 (3H, CH_3); ^{13}C-NMR (DMSO)-d_6) δ 185.8 (C-3), 173.1(-OCOCH_2CH_3), 168.3 (C-5), 155.4 (C-1), 128.4 (C-2), 124.2 (C-4), 100.3(C-9), 70.8 (C-11), 54.7 (C-10), 43.6 (C-16), 39.1 (C-8), 37.6 (C-14), 33.0 (C-6), 33.7 (C-13), 30.3 (C-12), 29.6 (C-15), 28.2 (-OCO<u>C</u>H_2CH_3), 25.2 (C-7), 24.8 (C-17), 18.9 (C-19), 11.3 (C-18), 9.4 (-OCOCH_2<u>C</u>H_3).

Results and discussion

When clobetasol was irradiated at 254 nm in argon flushed acetonitrile or in oxygen saturated solution it gave compound **7** as product. The photoreactions of **6** in 2-propanol at 254 nm followed a similar course of reaction under aerobic as well as anaerobic conditions. Irradiation at 310 nm either in argon saturated acetonitrile or 2-propanol gave product **7** along with a new compound **8**. At the same irradiation wavelength (310 nm) saturation of the solution with oxygen affected the product distribution: in 2-propanol **8** was not formed instead hydroperoxide **9** was obtained as main product along with **7**. Where as in acetonitrile a complex mixture of products was obtained (Scheme 3.6).

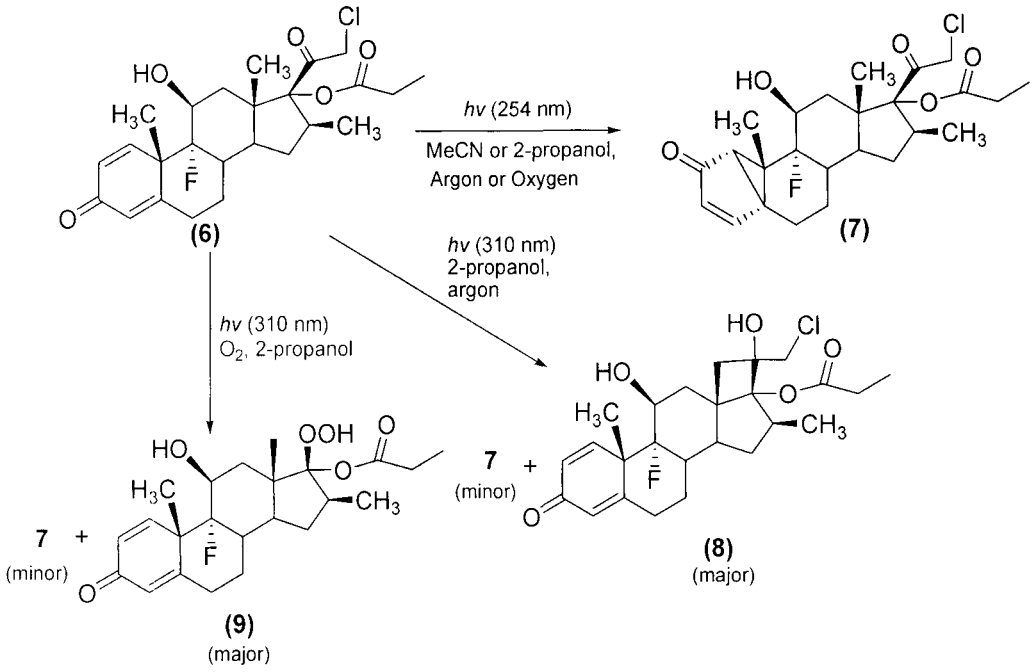
These results can be rationalized on the basis of different mechanism of photochemical reaction of the two-separated chromophores present in this drug. At 254 nm, cross-conjugated ketone absorbs predominantly or exclusively, which causes the well known

lumiketone rearrangement[24,25] of this chromophore and leads to the formation of compound **7** (Scheme 3.3). The rearrangement leading to **7** is a concerted process and therefore not affected by the solvent medium. On the contrary at 310 nm, where isolated ketone at C_{20} absorbs a large fraction of light, compound **8** was obtained as product, which arises via hydrogen atom abstraction from the close lying 18-methyl group followed by cyclization (Scheme 3.7). In oxygen saturated solution trapping of alkyl radical by oxygen is quite efficient to yield peroxy radical. This peroxy radical abstracts hydrogen from protic solvent (2-propanol) and gives the isolated hydroperoxy derivative **9** (Scheme 3.8).

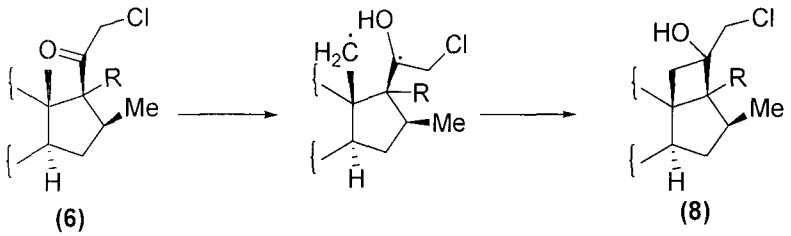
All the products obtained were characterized on the basis of the following spectral evidences. The IR spectrum of **7** showed absorption bands at 1350, 1161, 1030 (cyclopropyl), 1570 (C=C), 1675 (C=O). In the NMR spectrum of **7**, signals due to the rings B,C and D were found to be unaffected while signals due to ring A was strongly modified since only two of the olefinic CH were conserved and third was substituted by a sp^3 carbon. Two doublets centered at δ 6.66 and 5.68 with J=6.2 Hz in the ^{1}H-NMR spectrum and the IR band values indicated the presence of an α,β-unsaturated ketone in ring A. A proton singlet at δ 1.06 and ^{13}C-NMR signals at 40.4, 25.2 and 27.1 indicated a cyclopropyl carbonyl system in ring A. Proof of the stereochemistry came from a comparison of its circular dichroism spectra with those of other lumiketones that showed positive and negative cotton effects of similar magnitude and position to those reported in litrature[26,27] (Table 3.2).

Spectroscopic analysis of **8**, particularly NMR data indicated that the steroidal skeleton was unaffected but both the C_{20} ketone and 18-methyl signals were lacking. The presence of three deshielded olefinic protons at δ 7.34 (d, J=9 Hz, IH), 6.28 (d, J =9 Hz, 1H) and 6.09 (s, 1H) confirmed that the dienone system was intact, and on the basis of chemical shifts and spin-spin coupling constants these signals were assigned to the C-2, C-1 and C-4 protons respectively. Moreover, signals due to ring B, C and D were also unaffected. These data along with the appearance of a new methylene carbon and the IR absorption bands at 3410 (OH), 1680 (α,β-unsaturated C=O), 1630, 1615 (C=C) cm^{-1}, supported the assigned structure .

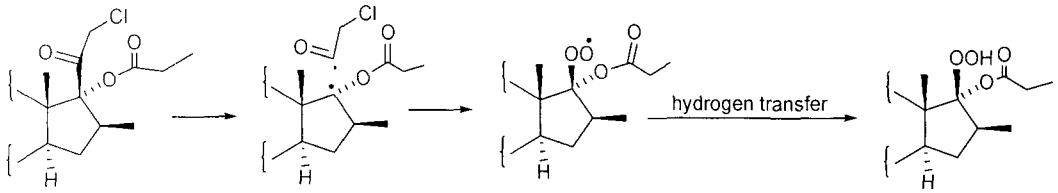
The NMR spectra of **9** suggested that the structural features in ring A, B and C were again conserved, while one of the side chains at C_{17} had been lost. In addition a strongly deshielded signal at δ 8.7 (brs, exch., 1H) in the ^{1}H-NMR and a new signal at δ 124.8 (C-17) in ^{13}C-NMR suggested the presence of hydroperoxy group in **9**. The spectroscopic indications (in the experimental section) allowed assignment of the hydroperoxide structure to **9**.


Cl
O
O
HO
CH3
O
H3C
CH3
F
O
(6)
hv (254 nm)
MeCN or 2-propanol,
Argon or Oxygen
(7)
hv (310 nm)
2-propanol,
argon
hv (310 nm)
O2, 2-propanol
OOH
7 +
(minor)
(9)
(major)
(8)
(major)

Scheme 3.6



Scheme 3.7



Scheme 3.8

Compd.	λ_{max} ($\Delta\varepsilon$)	λ_{max} ($\Delta\varepsilon$)	Crossover λ	λ_{max} ($\Delta\varepsilon$)	Crossover λ	λ_{max} ($\Delta\varepsilon$)
7		345 (-4.08)	315	280 (+12.22)	253	225 (-11.70)
Related lumiproducts[a]	355 (-3.71)	344.5 (-3.77)	311	275 (+10.3)	252	Short wavelength –ve CD

[a]see refs. [26, 27]

Table 3.2 Circular dichroism spectra of **7** and other related lumiproducts

References

1. J. Karoon, A. M. Oliver, M. N. Paddon-Row, J. W. Verhoven, *J. Am. Chem. Soc.*, 1990, **112**, 4868.
2. G. L. Closs, M. D. Johnson, J. R. Miller, P. Piotrowiak, *J. Am. Chem. Soc.*, 1989, **111**, 3751.
3. H. Morrison, *Rev. Chem. Intermed.*, 1987, **8**, 125.
4. Z. Z. Wu, J. Nash, H. Morrison, *J. Am. Chem. Soc.*, 1992, **114**, 6640.
5. Z. Z. Wu, H. Morrison, *J. Am. Chem. Soc.*, 1992, **114**, 4119.
6. H. Morrison, M. Pallmer, R. Loeschen, R. Pandey, K. Muthuramu, B. Maxwell, *J. Org. chem..*, 1986, **51**, 4676.
7. Z. Z. Wu, H. Morrison, *Photochem. Photobiol.*, 1989, **50**, 525.
8. A. Ricci, E. Fasani, M. Mella, A. Albini, *J. Org. chem.*, 2001, **66**, 8086.
9. A. Ricci, E. Fasani, M. Mella, A. Albini, *J. Org. chem.*, 2003, **68**, 4361.
10. T. Suzuki, T. Kato, T. Kitagaki, M. Ono, K. Shirakawa, M. Nagata, R. Konishi, *J. Toxicol. Sci.*, 1996, **21**, 475.
11. A. Keknes, P. Jahn, L. Lange, *J. Am. Acad. Dermatol.*, 1993, **28**, 786.
12. H. Uchiyama, T. Tanaka, N. Uehara, M. Nakamura, M. Tsuji, M. Shinomiya, H. Tanaka, *J. Toxicol. Sci.*, 1992, **3**, 283.
13. A. Albini and E. Fasani, Drugs: Photochemistry and Photostability, The Royal Society of Chemistry, Cambridge, 1998.
14. J. P. Tarayre, M. Aliaga, M. Barbara, J. Tisne-versailles, J. P. Couzinier, *Arzneimittelforschung* 1998, **38**, 542.

15. G. Rivara, R. R. Tomb, J. Foussereau, *Contact Dermatitis* 1989, **21**, 83.

16. J. Reisch, G. Enkel, N. Ekiz-Guecer and G. Nolte, *Liebigs. Ann. Chem.*, 1992, 63.

17. J. Reisch, Y. Topaloglu, G. Henkel, *Acta Pharm. Tech.*, 1986, **32**, 115.

18. M. Ogata, Y. Noro, M. Yamada, T. Tahara, T. Nishimma, *J. Pharm. Sci.*, 1998, **87**, 91.

19. T. Suzuki, T. Kato, T. Kitagaki, M. Ono, K. Shirakawa, M. Nagata, R. Konishi, *J. Toxicol. Sci.*, 1962, **2**, 475.

20. M. Takacs, N. Ekiz-Guecer, J. Reisch, A. Gergely-zobin, *Pharm. Acta Helv.*, 1991, **66**, 137.

21. N. Ekiz-Guecer, J. Reisch, G. Nolte, *J. Pharm. Biopharm.*, 1991, **37**, 234.

22. K. Thoma, R. Kerker, C. Weissbach, *Pharm. Ind.*, 1987, **49**, 961.

23. J. R. Williams, R. H. Moore, R. Li, C. M. Weeks, *J. Org. Chem.*, 1980, **45**, 2324.

24. K. Schaffner, M. Demuth, in Rearrangements in Ground State and Excited State, P. De Mayo Ed., Academic press, New York, 1980, vol 3, p 281.

25. D. Caine, W. M. Horspool, CRC Handbook of Organic Photochemistry and Photobiology, in P. S. Song Ed., CRC press, Boca Raton 1995, p 701.

26. J. Feri, C. Ganter, D. Kagi, K. Kocsis, M. Miljkovic, A. Siewinski, R. Wenger, K. Schaffner, O. Jeger, *Helv. Chim. Acta*, 1966, **49**, 1049.

27. K. Schaffner, G. Snatzke, *Helv. Chim. Acta*, 1965, **48**, 347.

28. C. A. Yang, S. I. R. William, I. A. MacFarlane, *British J. Clin. Pract.*, 1991, **45**, 61.

29. Martindale, Product monograph for clobitasol propionate, The Extra Pharmacopeia 31st ed. Royal Pharmaceutical Society, London, 1996, p 1093.

30. C. Dollerey, Product monograph for clobitasol propionate, Therapeutic Drugs, 2nd ed., Churchi 11 Livingstone, London, 1999, p 266.

Chapter 4

Photochemical Studies on Flutamide

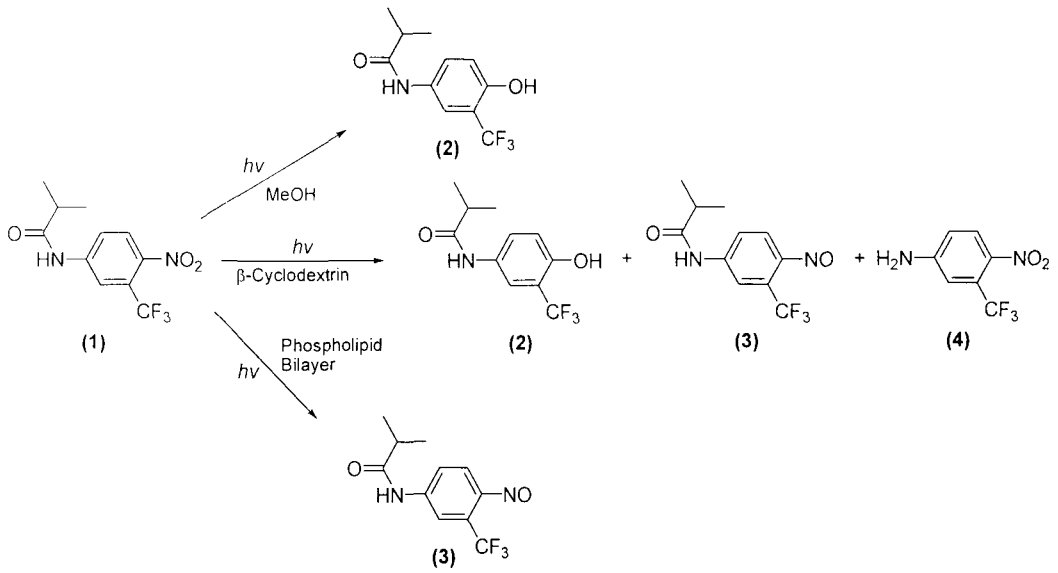
Introduction

Flutamide (2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide), **1, FM)** is a non-steroidal androgen drug which blocks androgen receptor sites and is used in advanced prostate cancer.[1-5] Recent reports have shown that use of this drug is associated with photosensitivity reaction and can induce adverse biological effects such as sun-exposed eruption in patients after drug treatment.[6-10] The photochemistry of **FM** in homogeneous media has been characterized by low photoreactivity as well as by very low efficiency in photosensitizing singlet oxygen formation.[11-13] The photoreactivity of free molecule has been rationalized on the basis of an intermolecular nitro to nitrite rearrangement followed by cleavage of nitrite intermediate leading to phenol derivative **2** as the main stable product. The out of plane geometry of the nitro group and the consequent overlap of the p-orbital of oxygen with the adjacent orbital of the aromatic ring is prerequisite to observe such type of intramolecular photorearrangements. The photolysis of **FM** in presence of biological mimic systems such as cyclodextrin, micelles and phospholipid bilayers has been shown to dramatically increase the photodecomposition and the different photodegradation pathways were observed.[12,13] (Scheme 4.1)

Herein we have investigated:

[A] Photolysis of flutamide adsorbed on silica on the silica gel TLC plates.

[B] Photostability determination of commercially available flutamide oral dosage formulations.



Scheme 4.1

[A] Photolysis of flutamide adsorbed on silica on the silica gel TLC plates.

Photochemical investigation of phototoxic drugs in biologically mimicking systems is becoming an active area in research, because real life processes occur at surfaces, interfaces and multiphase heterogeneous systems. Therefore, to present a model for a close correlation between phototoxic and photochemical behaviour of the drug *in vivo*, it is significant to investigate drug photobehaviour in biologically mimicking systems. Interface chemistry has played an important role in industrial and natural processes, a common feature of this chemistry is the existence of an interface that provides an extensive surface whose chemical and geometrical features lead to optimum reaction rates and the high chemical selectivity.[14] With this interest, herein, we have investigated photolysis of flutamide adsorbed on silica on the silica gel TLC plates, a biologically mimic system, similar to situation in liposomes.[15]

Experimental

Instrumentation

The photochemical irradiation was carried out by using a medium pressure mercury vapour lamp (125 W). The light was passed through a pyrex glass filter. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 spectrometer using $SiMe_4$ as internal standard. FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using *m*-nitrobenzyl alcohol (NBA) matrix and argons as FAB gas. Merck silica gel 60 F_{254} was used for preparing photoirradiation plates and column chromatography was performed on Merck silica gel 60 (70-230 mesh).

Chemicals and reagents

All chemicals used were of analytical grade. Flutamide was extracted from commercial medicinal product Cytomid-250 (Cipla Ltd., Mumbai, India) with a soxhlet extractor using benzene as a solvent and recrystallized from the same solvent. The purity of the crystallized drug was checked by TLC.

Photoirradiation procedure

Flutamide solution in methanol was misted on silica gel TLC plates by using a TLC reagent sprayer and then irradiation of these TLC plates was carried out with UV lamp under a stream of nitrogen. After the photolysis, the plates were scarped and adsorbate was extracted with methanol and filtered. The analysis of product was performed by the thin layer chromatography (TLC), on a precoated silica gel TLC plates using dichloromethane: cyclohexane 80:20 (vol/vol), which showed the photoproduct **3** (R_f = 0.30) and the starting compound (R_f = 0.20). The solvent was removed in a rotary evaporator at room temperature (30° C) and the crude product so obtained, was purified by silica gel column chromatography, which yielded 2-methyl-N-[4-nitroso-3-(trifluoromethyl) phenyl] propanamide **(3)** as product.

Characterization of products

2-Methyl-N-[4-nitroso-3-(trifluoromethyl) phenyl] propanamide (3): ^{1}H-NMR (CD_3OD) δ 8.32 (d, J= 2.4 Hz, 1H, H-2), 7.94 (dd, J= 8.5 Hz, 1H, H-6), 6.28 (d, J=8.5 Hz, 1H, H-5), 2.58 (m, 1H, $CH(CH_3)_2$), 1.18 (6H, $CH(CH_3)_2$); ^{13}C-NMR (CD_3OD) δ 181.0 (CONH), 162.2 (C-4), 138.5 (C-1), 131.9 (C-3), 124.0 (CF_3), 122.6 (C-6), 112.7 (C-2), 110.4 (C-5), 31.3 ($CH(CH_3)_2$), 18.6 ($CH(CH_3)_2$); FAB-MS: m/z (rel. int.): 261

($[C_{11}H_{11}F_3N_2O_2+H]^+$, 9), 230 ($C_{11}H_{11}F_3NO^+$, 15), 217 ($C_8H_4F_3N_2O_2^+$, 10), 189 ($C_7H_4F_3N_2O^-$, 18), 174 ($C_7H_3F_3NO^+$, 100), 145 ($C_7H_4F_3^+$, 20), 86 ($C_4H_8NO^+$, 25).

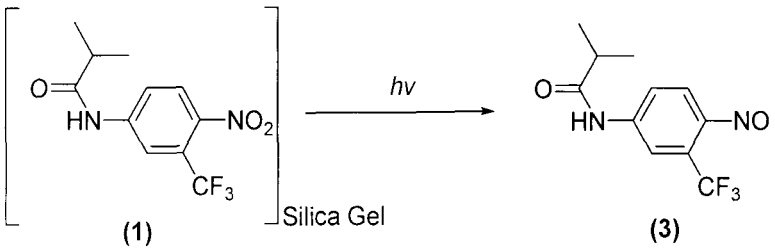
Results and discussion

Irradiation of silica gel TLC plates misted with flutamide under UV light and a stream of nitrogen afforded the photoproduct **3** as the only major isolated product (Scheme 4.2). The structure of photoproduct **3** was confirmed on the basis of IR, NMR and mass spectral studies (experimental section). A similar photobehaviour of **FM**, after its incorporation to β-cyclodextrin cavities and in unilamillar phospholipid bilayer vesicles has been well characterized by Sortino et al.[12] The photoreactivity of this drug in homogenous media is exclusively characterized by nitro to nitrite rearrangement leading to phenol derivative **2** as the main stable photoproduct (Scheme 4.1). The photolysis of **FM** after its incorporation into β-cyclodextrin cavities increases the photodecomposition with the photogeneration of photoproduct **2** along with **3** and **4**, whereas in phospholipid bilayers photoproduct **3** was noticed as the sole stable photoproduct. In this study, for the photolysis of **FM** on silica gel we have also isolated photoproduct **3** as the only product. A possible hypothesis to explain the photogeneration of **3** and inhibition of **2** may be due to the structural changes of **FM** occurring upon its incorporation in silica gel cavities.

It is generally accepted that the structure of the surface of silica consists of interconnected network of silanol (SiOH) and siloxane (SiOSi) linkage.[16] Silanol linkage are generally classified as (1) the isolated or free (non-hydrogen bonded silanols that are characteristic of crystalline silica) (2) the vicinal or hydrogen bonded or active

silanols that are characteristic of amorphous silica and (3) hydrated silanols that may be the result of water binding to either free or vicinal silanols (Figure 4.1). The active silanols are so positioned on the silica surface so as to permit hydrogen bonding. In this regard, perpendicular geometry of nitro group with respect to aromatic ring, which is necessary for the formation of **2**, may be lost due to weak interactions involving active silanol and CF_3/NO_2 groups present in flutamide, and would account well for the obtained results.

Our results concerning to photobehaviour of **FM** on silica gel further support the fact that the drug photochemical behaviour in homogeneous media is the first step for understanding of molecular basis of the drug but alone can not be used to understand *in vivo* photochemistry. For a correlation between photobehaviour and phototoxicity of the drugs, its photoreactivity should also be investigated in heterogeneous systems of increasing complexicity, specific interactions and steric constraints.



Scheme 4.2

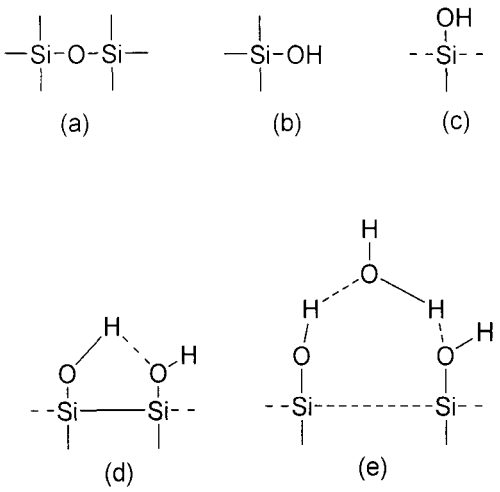


Figure 4.1 The chemical composition of silica surface (a) Silioxane (b) Silanol (c) Isolated silanol (d) Vicinal silanol (e) Hydrated silanols.

[B] Photostability determination of commercially available flutamide oral dosage formulations.

Some drugs on exposure to light undergo important chemical changes accompanied by alternation in their activities, and in some cases there may be a total loss of the therapeutic activity. Manufacturers of pharmaceutical products use light resistant coating/or packing to minimize their photodegradation. Long term exposure to sunlight or artificial light may result in photodamage, if pharmaceutical formulations are improperly stored, and thus poor storage conditions may potentially decrease clinical efficiency of these products. Differences in the degree of light protection may also exist between different formulation types. Consequently, if differences in photostability between these formulations are present, product substitution may not be warranted.

With this interest, herein, we have investigated the photostability of commercially available flutamide oral dosage formulations (tablets) available in India. We have also compared the photodegradation of **FM** powder and methanol solution of three commercially available **FM** oral dosage formulations.

Experimental

Chemicals and reagents

All chemicals used were of analytical or HPLC grade. Pure flutamide powder was extracted from commercial medicament Cytomid-250 (Cipla ltd., Mumbai, India) with a soxhlet extractor using benzene as a solvent and recrystallized from the same solvent. The purity of the crystallized drug was checked by TLC. Three **FM** tablets (250 mg)

[Cytomid-250 (Cipla Ltd.), Plutamide (Torrent Pharm. Ltd.), Prostamid (BDH Industries Ltd.)] obtained from local market in India were studied. 2-hydroxy-5-nitrobenzaldehyde was used as internal standard (IS).

The **FM** stock solution was prepared in methanol (10 mg/ml) and standard solutions were obtained by serial dilution. The Internal standard solution (IS) was prepared (40 µg/ml) in methanol. 1 ml of IS solution was mixed with 1 ml of the tested concentrations of **FM** (12.5–150 µg/ml) and 20 µl of the mixed solution was injected to HPLC.

Irradiation test

For artificial light irradiation, a 40 W tungsten lamp was used. **FM** samples were placed 50 cm from the lamp in a cabinet. Protected samples from extraneous light were placed in aluminum foils. Exposure to indirect sunlight was also used to compare the efficacy of artificial light and natural room daylight in photodecomposition of **FM**. Samples were irradiated from 0-12 weeks in artificial light and indirect sunlight. Samples were collected at 0, 1, 2, 4, 6, 8, 10 and 12 weeks intervals (n=3). **FM** powder samples (10 mg) were placed in 10 ml clean glass vials, irradiated from 0-12 days and samples were collected at 0, 1, 2, 3, 7, 10 and 12 days. Also a total of 11 × 1 ml methanolic **FM** solution samples (10 µg/ml) were placed in 5 ml clear glass vials and irradiated for a period of 0-360 min. Samples were taken at 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360 mins.

Sample preparation

Flutamide tablets: Five irradiated **FM** tablets were crushed into fine particles and a quantity equivalent to 10 mg of **FM** was placed in a centrifuge tube and 2 ml of chloroform was added. The mixture was vortexed for 30 s and centrifuged for 5 min. Twenty µl of supernatant was drawn by Hamiltonian syringe and added to a tube containing 100 µl of IS solution (40 µg/ml). This solution was evaporated to a dry residue under Nitrogen stream. Two hundred µl of mobile phase was added to the residue and vortexed for 10 s. Aliquots of 10 µl were injected to HPLC.

Flutamide powder samples: Each of the irradiated **FM** powder samples were diluted with 2 ml of chloroform and vortexed for 20 s. Twenty µl of this solution was mixed with 100 µl of IS solution (40 µg/ml), and dried over nitrogen stream, then 1 ml of mobile phase was added and vortexed for 10 s, Finally, 10 µl of this solution was injected to HPLC.

Flutamide methanolic solution: One hundred µl of IS was added to the vials containing 1 ml of **FM** (100 µg/ml) and vortexed, and then 10 µl of the solution was injected to HPLC.

Identification of the photoproduct

0.2 mg/ml of **FM** methanolic solution was exposed to indirect sunlight. At 5 min intervals 10 µl of the solution were injected to HPLC until no **FM** peak was observed in

chromatogram (45 min). The solution was then evaporated over nitrogen stream and subjected to mass spectrometry after purifying by using column chromatography.

Chromatography and instrumentation

FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using *m*-nitrobenzyl alcohol (NBA) matrix and argons as FAB gas. Merck silica gel 60 F_{254} plates were used for photoirradiation and column chromatography was performed on Merck silica gel 60 (70-230 mesh). HPLC was performed on a Hewlett-Packard 1100 chromatograph equipped with online photodiode array detector. The analysis of **FM** and its photoproducts was achieved on a LiCharoCart RP-18 column (5 μm packing, 4× 250 mm) eluting with a linear gradient of CH_3CN at a flow rate of 1 ml/min with monitoring at 300 nm.

Results and discussion

In the present study photostability of **FM** formulations available in India was determined after exposure to indirect natural light and continuous artificial light. **FM**, its photoproduct **2** and IS were eluted at approximately 8.8, 6.5 and 4.9 min respectively. Resolution between **FM** and **2** was adequate and no buffer and pH adjustment was required with this chromatographic system. Mass spectra of photodecomposition product produced major fragments at 248 (M^{+1}, 4%), 247 ($M^{+\cdot}$ 30 %), 177 (M^+-$(CH_3)_2CHCO$, 100%), 157 (50%), 71 (20%) that confirmed the structure of photoproduct **2**.

Figure 4.2 shows a typical chromatogram of (a) commercial 10 mg **FM** tablet irradiated for 12 weeks with indirect natural sunlight (b) a sample of **FM** methanolic solution irradiated with indirect natural sunlight for 6 hours and (c) a sample of **FM** methanolic solution irradiated with artificial light for 6 hours.

Figure 4.3a shows photodegradation plots of **FM** powder and figure 4.3b shows photodegradation of **FM** methanolic solution after artificial light irradiation. Photodegradation of **FM** powder, measured as percentage loss of **FM** powder exceeds 10% in 30 hours. Photodegradation of **FM** methanolic solution exceeds 10% in approximately 45 min. and was essentially complete within 7 hours. It was also observed that the efficacy of artificial light in photodecomposition of **FM** is more than natural indirect sunlight and it can be due to the higher intensity of artificial light than natural light. The percentage of **FM** content (w/w initial **FM** content) was also measured in all three tested formulations irradiated for up to 12 weeks by artificial and natural light.

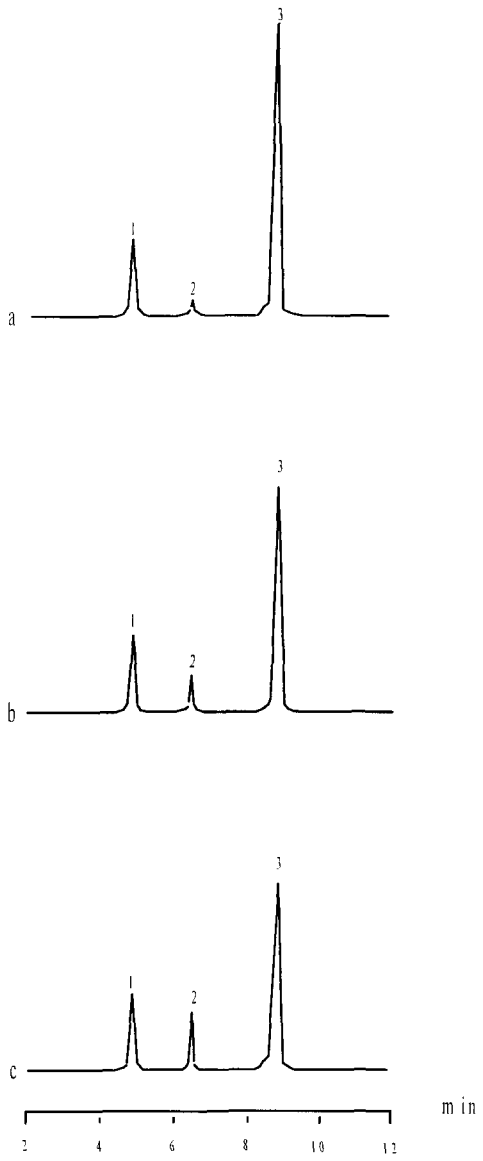


Figure 4.2 chromatogram of (a) commercial 10 mg **FM** tablet irradiated for 12 weeks with indirect natural sunlight (b) a sample of **FM** methanolic solution irradiated with indirect natural sunlight for 6 hours and (c) a sample of **FM** methanolic solution irradiated with artificial light for 6 hours; 1=IS, 2= photoproduct **2**, 3=**FM**.

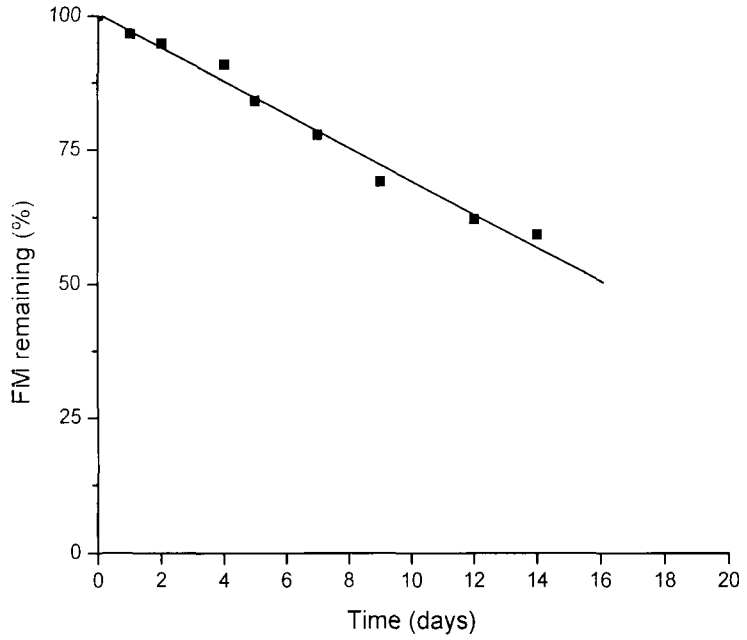


Figure 4.3a Photodecomposition of **FM** powder by using artificial light

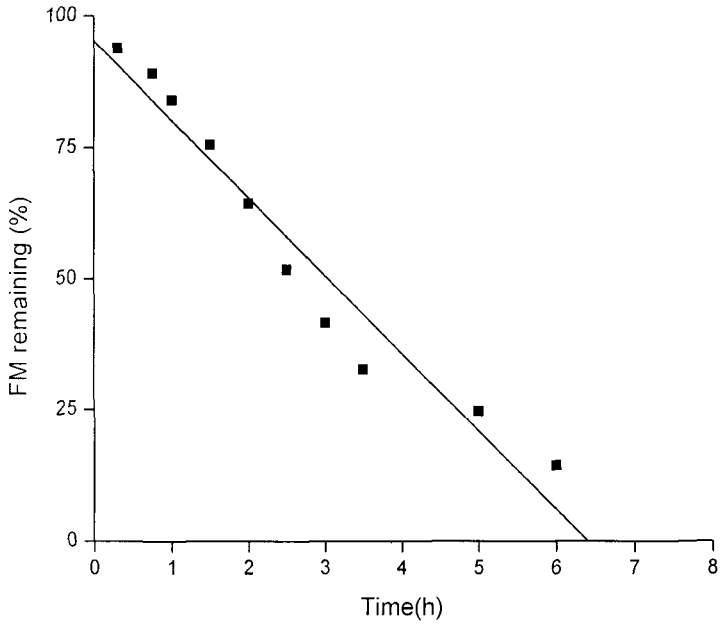


Figure 4.3b Photodecomposition of **FM** methanolic solution using artificial light

Table 1 summarizes photodegradation data for each formulation after 0, 5 and 12-week exposure to artificial light. The average percentage of photoproduct **2** found in the formulations was 1.05, 1.21, 1.49 for 0, 5, and 12 weeks, respectively. Results obtained in this study showed that differences between data obtained were not significant and none of the tested FM formulations underwent any appreciable decomposition (> 10%), even after 12 weeks irradiation.

	Percentage of photoproduct **2** (w/w initial FM content)		
Formulation	0 week	5 week	12 week
Cytomid-250	1.0	1.14±0.08	1.28±0.11
Prostamid	1.0	1.10±0.03	1.39±0.24
Plutamide	1.15	1.40±0.13	1.81±0.19

Table 1 Flutamide formulations photodegradation data

References

1. D. K. Ornstein, *Urology*, 1996, **48**, 901.
2. E. J. Small, *Curr. Opin. Oncol.*, 1997, **9**, 277.
3. R. Dacker, *Radiol. Technol.*, 1997, **68**, 194.
4. C. Mahler, J. Verhelst, L. Denis, *Clin. Pharmacolinet.*, 1998, **34**, 405.
5. L. Boccon-Gibod, *Eur. Urol.*, 1998, **33**, 159.
6. D. Leroy, A. Dompmartin, C. Szezurko, *Photodermatol. Photoimmunol. Photomed.*, 1996, **12**, 216.
7. I. Moraillon, M. Jeammougin, J. R. Manciet, J. Revuz, M. Bagot, *Photodermatol. Photoimmunol. Photomed.*, 1991, **8**, 264.
8. J. Vilaplana, C. Romaguera, A. Azon, M. Lecha, *Contact Dermatitis*, 1998, **38**, 68.
9. R. Yokote, Y. Tokura, N. Igarashi, O. Ishikawa, Y. Miyachi, *Eur. J. Dermatol.*, 1998, **8**, 427.
10. M. B. Reid, L. M. Glode, *J. Urol.*, 1998, **159**, 2098.
11. F. Vargas, C. Rivas, H. Mendez, A. Fuentes, G. Fraile, M. Velasquez, *J. Photochem. Photobiol: B. Biol.*, 2000, **58**, 108.
12. S. Sortino, G. Marconi, G. Condorelli, *Chem. Commun.*, 2001, 1226.
13. S. Sortino, S. Giuffrida, G. De. Guidi, R. Chillemi, S. Petralia, G. Marconi, G. Condorelli, S. Sciuto, *Photochem. Photobiol.*, 2001, **6**, 73.
14. G. A. Somorjai, in Chemistry in Two Dimensions: Surfaces, Cornell University Press, Ithaca, 1981.

15. J. K. Yamamoto, R. F. Borch, *Biochemistry*, 1985, **24**, 3338.

16. N. J. Turro, *Tetrahedron Lett.*, 1987, **43**, 1589.

Chapter 5

Photooxygenation Studies on Furanoterpenes

Introduction

For over a century, natural products have served as tools and leads for the developments of new drugs, and several natural compounds from plants and animals kingdom are now useful drugs. Moreover, plenty of plant materials for their biologically active principles have proved to be of potential medicinal value.[1-4] The photoreactivity of synthetic drugs have been intensively studied in the recent past for their photosensitizing properties, phototoxicity and phototherapeutic values and also for photodegradation studies.[5-8] However, a significant and related work on photochemistry of medicinally and biologically active compounds from plants is sporadic.[9-12] It is of importance to study the photoreactivity of biologically active plant metabolite for a correlation to their possible *in vivo* photoreactions and phototoxicity. Several natural plant extracts containing terpenoids are widely used in agriculture and medicine.[13,14] Photochemical study is expected to through light on improving the stability of these compounds into the biological extracts containing terpenoids. Moreover, the significance of generation and reactions of singlet oxygen with biomolecules in plants and living systems have been recognized.[15] It is now generally accepted that certain secondary plant substances have a defensive role, offering protection against predators, pathogens and competitors. It is increasingly recognized that certain of these defensive chemicals are capable of photosensitizing reactions that involve the transfer of light energy to oxygen. It is thus apparent that plants may utilize these activated forms of oxygen, such as

singlet oxygen, in their own defense. Other secondary plant products may have a physiological role in that they protect the plant against damaging photodynamic reactions by quenching the excited singlet state of oxygen.

Within the context we have investigated photooxidation reactions of the following terpenoids:

[A] Sensitized photooxygenation of tinosponone, a clerodane diterpene from *Tinospora cordifolia*.

[B] Photooxidation of 2β-angeloyloxy-10β-H-furanoeremophilane.

[A] Sensitized photooxygenation of tinosponone, a clerodane diterpene from *Tinospora cordifolia*

Tinosponone **(1)**, a clerodane diterpene isolated from *Tinospora cordifolia*, a plant of recognized medicinal values which is widely used as anti-bacterial, analgesic, antipyretic and also for the treatment of jaundice, skin diseases, diabetes, anaemia etc.[16-20] Several compounds containing 3-substituted furan moiety have been isolated from this plant species.[21-23] In spite of immense medicinal use of this plant extract the photochemical sensitivity of their bioactive constituents has not been described in the literature. The 3-substituted furan moiety is quite susceptible to attack by biological oxygens; we therefore, have investigated photooxygenation of tinosponone under different combinations of sensitizer dyes and solvents.

Experimental

Apparatus and chemicals

Irradiations were carried out in a photoreactor equipped with medium pressure mercury vapour lamp inserted in a water-cooled immersion well with continuous supply of water. IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RX1. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX-300 spectrometer using $SiMe_4$ as internal standard and $CDCl_3$ as solvent. FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using *m*-nitrobenzyl alcohol (NBA) matrix and argons as FAB gas. Elemental analyses were carried on a Carlo Erba

model 1108 Elemental analyzer. High-resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer. All solvents and chemicals used were of analytical grade. Tinosponone was isolated from stem of *Tinospora cordifolia* according to literature procedure.[24] The purity of **1** was determined by comparison of its melting point and ^{1}H-NMR with that of literature value. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70-230 mesh).

Irradiation procedure

An air-saturated benzene solution of tinosponone **(1)** (100 mg, 1.5 mM in 200mL) was irradiated for 8 hr with a medium pressure mercury vapour lamp (125 W) in presence of methylene blue (0.01 gm, 10% wt/wt of tinosponone). Complete decomposition of **1** was monitored by thin layer chromatography (ethyl acetate: hexane; 3 : 7). Removal of the solvent under reduced pressure and column chromatography of the resulting photoproduct on silica gel yielded compound **2** and **3**. This photoreaction was also carried out under nitrogen atmosphere by saturating the solution with nitrogen prior to irradiation and with continuous bubbling during irradiation.

Similar experiments were carried out by using different combinations of solvents and sensitizers (Table 5.1 and 5.2). Two different sets of reactions were also carried out in similar way by using DABCO/sodium azide (10% wt/wt of tinosponone) with methylene blue as sensitizer.

Characterization of products

(3S,4aS,4bS,8R,8aR,10aR)-8-Hydroxy-3-(5'-hydroxy-2'-oxo-2',5'-dihydrofuran -3'-yl)-4a,8a-dimethyl-3,4,8,8a,9,10-hexahydro-10aH-benzo[f]isochromene-1,5 (4aH,4bH)-dione (2): mp 177°C; R_f 0.83; $[\alpha]^{23}_D$ –93.7 (c 0.92, $CHCl_3$); HRMS: [M^+] calcd for $C_{19}H_{22}O_7$, 362.3738; found, 362.3731; IR (KBr) *v* 3500, 3390, 1745, 1715, 1678, 1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.94 (d, 1H, J=7.1 Hz, H-4`), 6.67 (dd, 1H, J=10.8, 5.2 Hz, H-7), 6.15 (d, 1H, J=7.0 Hz, H-5'), 5.91 (d, 1H, J=10.8 Hz, H-6), 4.91 (dd, 1H, J=12.2, 3.6 Hz, H-3), 4.82 (brs, exch., -OH), 4.28 (d, 1H, J=5.1 Hz, H-8), 2.42 (dd, 1H, J=14.8, 3.4 Hz, H-4), 2.38 (brs, 1H, H-10a), 2.21 (m, 1H, H-9), 2.20 (brs, 1H, H-4b), 2.18 (m, 1H, H-10), 1.65 (dd, 1H, J=15.4, 12.2 Hz, H-4), 1.61 (m, 1H, H-10), 1.42 (s, $-CH_3$), 1.1 (dt, 1H, J=13.8, 4.5 Hz, H-9), 0.80 (s, $-CH_3$); ^{13}C-NMR ($CDCl_3$, δ, ppm): 204.5 (C-5), 176.2 (C-1), 175.7 (C-3), 141.5 (C-1), 143.1 (C-7), 136.5 (C-2), 127.5 (C-6), 97.5 (C-10), 74.5 (C-8), 60.5 (C-3), 52.6 (C-4b), 49.2 (C-10a), 42.9 (C-8a), 40.1 (C-4), 34.8 (C-4a), 30.9 (CH_3), 29.9 (C-9), 26.6 (CH_3), 19.2 (C-10); MS m/z (relative intensity): 345 ($C_{19}H_{21}O_6^+$, 14), 318 ($C_{18}H_{22}O_5^+$, 100), 303 ($C_{18}H_{23}O_4^+$, 15), 274 ($C_{17}H_{22}O_3^+$ 10), 263 ($C_{15}H_{19}O_4^+$, 17), 261 ($C_{17}H_{25}O_2^+$, 9); Anal. calcd. for $C_{19}H_{22}O_7$: C 62.97, H 6.12, O 30.91; found C 62.84, H 6.08, O 30.98.

(3S,4aS,4bS,8R,8aR,10aR)-8-Hydroxy-4a,8a-dimethyl-3-((1'R)-3'-oxo-4',6'-dioxa-bicyclo[3.1.0]hexan-1'-yl)-3,4,8,8a,9,10-hexahydro-10aH-benzo[f] isochromene-1,5(4aH,4bH)-dione (3): mp 164°C; R_f 0.62; $[\alpha]^{23}_D$ –84.6 (c 0.47, $CHCl_3$); HRMS: [M^+] calcd for $C_{19}H_{22}O_7$, 362.3738; found, 362.3751;

IR (KBr) ν 3430, 1750, 1710, 1660, 1210, 950, 745 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.52 (dd, 1H, J=10.6, 4.8 Hz, H-7)), 5.75 (d, 1H, J=10.4 Hz, H-6), 5.23 (s, 1H, H-4), 4.87 (dd, 1H, J=12.4, 3.8 Hz, H-3), 4.30 (d, 1H, J=5.1 Hz, H-8), 4.12 (brs, exch., -OH), 2.28 (dd, 1H, J=14.2, 2.9 Hz, H-4), 2.22 (brs, 1H, H-10a), 2.36 (m, 1H, H-9), 2.2 (s, 1H, H-1), 2.16 (brs, 1H, H-4b), 1.98 (m, 1H, H-10), 1.71 (m, 1H, H-10), 1.58 (dd, 1H, J=15.3, 11.8 Hz, H-4), 1.38 (s, CH_3), 1.12 (dt, 1H, J=13.6, 4.4 Hz, H-9), 0.80 (s, CH_3); ^{13}C-NMR ($CDCl_3$, δ, ppm): 202.6 (C-5), 175.2 (C-1), 171.1 (C-2), 145.2 (C-7), 130.7 (C-6), 120.4 (C-4), 75.2 (C-8), 59.2 (C-10), 56.8 (C-3), 52.5 (C-4b), 48.1 (C-10a), 44.2 (C-8a), 41.7 (C-4), 36.6 (C-4a), 36.2 (C-1), 31.3 (CH_3), 25.4 (CH_3), 18.8 (C-10), 18.6 (C-9),; MS m/z (relative intensity): 318 ($C_{18}H_{22}O_5^+$, 20), 290 ($C_{17}H_{22}O_4^+$, 100), 278 ($C_{15}H_{18}O_5^+$, 11), 276 ($C_{17}H_{24}O_3^+$, 11), 263 ($C_{15}H_{19}O_4^+$, 9), 221 ($C_{14}H_{21}O_2^+$, 16), 84 ($C_4H_4O_2^+$, 19); Anal. calcd. for $C_{19}H_{22}O_7$: C 62.97, H 6.12, O 30.91; found C 62.74, H 6.34, O 31.05.

(3S,4aS,4bS,8R,8aR,10aR)-8-Hydroxy-3-(5'-hydroperoxy-2'-methoxy-2',5'-dihydrofuran-3'-yl)-4a,8a-dimethyl-3,4,8,8a,9,10-hexahydro-10aH-benzo[f] isochromene-1,5(4aH,4bH)-dione (4): mp 169°C; $[\alpha]^{23}_D$ –77.8 (c 0.34, $CHCl_3$); HRMS: [M^+] calcd for $C_{20}H_{26}O_8$, 394.4156; found, 394.4152; IR (KBr) ν 3520, 3500, 1670, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.12 (brs, exch., OOH), 6.61 (dd, 1H, J=10.7, 5.2 Hz, H-7), 6.21 (d, 1H, J=7.4 Hz, H-10), 5.84 (s, 1H, H-3), 5.72 (d, 1H, J=7.2 Hz, H-1), 5.62 (d, 1H, J=10.8 Hz, H-6), 4.94 (dd, 1H, J=12.1, 3.2 Hz, H-3), 4.64 (brs, exch., -OH) 4.26 (d, 1H, J=4.8

Hz, H-8), 3.22 (s, 3H, -OCH_3), 2.48 (dd, 1H, J=13.8, 3.2 Hz, H-4), 2.30 (brs, 1H, H-10a), 2.22 (m, 1H, H-9), 2.20 (brs, 1H, H-4b), 2.12 (m, 1H, H-10), 1.68 (dd, 1H, J=15.2, 12.8 Hz, H-4), 1.65 (m, 1H, H-10), 1.48 (s, 3H, CH_3), 1.1 (dt, 1H, J=13.8, 4.0 Hz, H-9), 0.82 (s, 3H); ^{13}C-NMR ($CDCl_3$, δ, ppm): 200.5 (C-5), 173.4 (C-1), 143.7 (C-7), 141.4 (C-2), 129.4 (C-6), 114.7 (C-1), 114.7 (C-1), 112.2 (C-10), 98.4 (C-3), 73.8 (C-8), 58.1 (C-3), 53.8 (OCH_3), 51.3 (C-4b), 47.6 (C-10a), 43.1 (C-8a), 39.2 (C-4a), 33.7(C-CH_3), 26.1 (C-9), 25.2 (CH_3), 18.4 (C-10); MS m/z (relative intensity): 301 ($C_{18}H_{21}O_4^+$, 100), 363 ($C_{19}H_{23}O_7^+$, 18), 361 ($C_{20}H_{25}O_6^+$, 21), 331 ($C_{19}H_{23}O_5^+$, 13), 288 ($C_{17}H_{20}O_4^+$, 11), 206 ($C_{14}H_{22}O^+$, 15), 84 ($C_4H_4O_2^+$, 21); Anal. calcd. for $C_{20}H_{26}O_8$: C 60.90, H 6.64, O 32.45; found C 60.98, H 6.45, O 32.52.

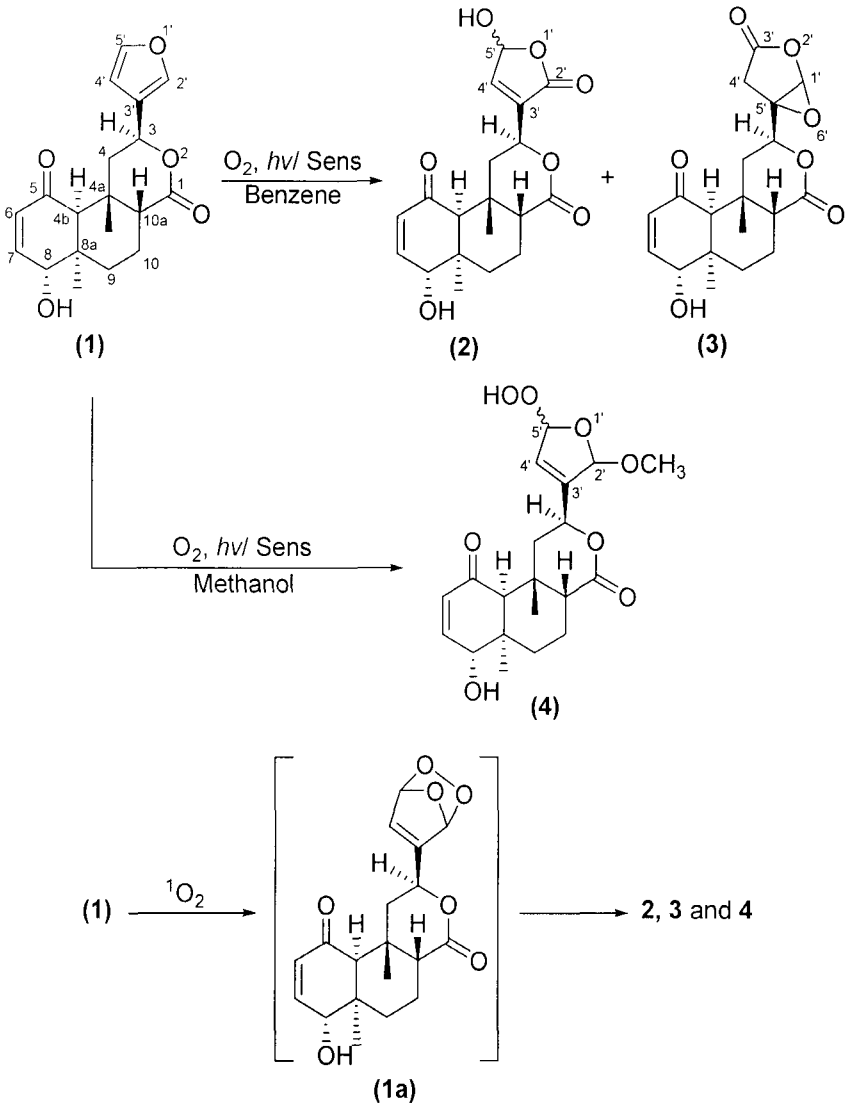
Results and discussion

Irradiation of air-saturated benzene solution of tinosponone with methylene blue as sensitizer in a water-cooled immersion well type photoreactor equipped with medium pressure mercury vapour lamp and purification of the crude product by silica gel column chromatography afforded two compound **2** (epimeric mixture) and **3.** When tinosponone was irradiated with methylene blue in methanol, the chromatographic analysis (TLC) of irradiated mixture did not show the presence of any of the previously identified products (**2** and **3**), rather a new product **4** (epimeric mixture) was observed (Scheme 5.1). When these photoreactions were carried out in

the absence of sensitizer same products were obtained but the reaction was observed to be slow.

The effect of the nature of solvent on photooxidation was studied by using different solvents. The amount of substrate could not be kept same, as the solubility of substrate was different in different solvents. Therefore, relative yields of products were determined in these cases. For this purpose, different reaction mixtures were irradiated under standard condition for the same time period. Then 15 ml of each solution was taken out, concentrated and subjected to preparative TLC for the isolation of the products, and correlation of their yields. Yields of products in different solvents were found to vary with the polarity of the solvent. The yield was higher in polar solvents in comparison to non-polar solvents (Table 5.1). This observation may be attributed to longer lifetime of $^1O_2$ in polar solvents.[25,26] Owing to the solubility problem, the concentration of **1** was not same in all the solutions, as it was in the case of methylene blue therefore, possibility of energy transfer for different yields of products cannot be discarded. To confirm whether energy transfer or longer lifetime of $^1O_2$ is responsible for different yields of products, we conducted experiments by varying the concentration of sensitizer (5×10^{-3} to 2×10^{-2} mol L^{-1}) to the concentration of tinosponone in different solvents. Similar pattern of product formation was also obtained in these cases, which supports the fact that lifetime of $^1O_2$ and in turn polarity of solvent is responsible for the observed difference in the

yields. The dependence of percentage yields of the products on triplet energies of various sensitizers has also been studied. It was observed that rose bengal and methylene blue was much more efficient than riboflavin and benzophenone in the photosensitized decomposition of **1** (Table 5.2). This may be due to the fact that rose bengal and methylene blue, with lower triplet energies, produce singlet oxygen in large amount[27,28] by type II mechanism.[29]



Scheme 5.1

Solvent	Lifetime of $^1O_2$ [(a)] (μs)	Yields of products (%)[b] (**2**+**3**)
Benzene	24	32.3 (19.6+12.7)
Acetone	26	31.6 (18.9+12.7)
Acetonitrile	30	35.2 (22.8+12.4)
Chloroform	60	40.4 (27.1+13.3)

Concentration of tinosponone =100 mg/200mL, 1.5 mM. Concentration of methylene blue = 10% wt/wt of tinosponone. Time of irradiation = 4 hours. [a]See refs. [25, 26]. [b]Yields of the products were determined after isolation according to experimental part.

Table 5.1 Yields of products, by methylene blue photosensitized reaction of tinosponone with different solvents

Sensitizer	Triplet energy[a] (Kcal/mole)	Yields of products (%)[b] (**2**+**3**)
Methylene blue	33.5 – 34.0	31.7 (19.1+12.6)
Rose bengal	39.2 – 42.2	30.3 (17.1+13.2)
Riboflavin	57.8	21.2 (11.3+9.9)
Benzophenone	68.6 – 69.1	19.9 (9.9+10.0)

Concentration of tinosponone =100 mg/200mL, 1.5 mM. Concentration of dye = 10% wt/wt of tinosponone. Time of irradiation = 4 hours. [a]See ref. [35]. [b]Yields of the products were determined after isolation according to experimental part. Benzene was used as solvent.

Table 5.2 Effect of triplet energies of different sensitizers on the yields of products.

On other hand riboflavin and benzophenone (higher triplet energies) act mainly by type I photosensitized photooxidation, do not produce significant amount of $^1O_2$.[30] The participation of $^1O_2$ in the reaction was confirmed by studying the effect of some scavengers on the yield of this photooxidation reaction. The drastic lowering of the yields of the products in presence of scavengers (DABCO-17%; sodium azide-14%) confirms that $^1O_2$ is active oxidizing species in this photoreaction. Also no reaction was observed on conducting experiments under nitrogen atmosphere. When irradiations were carried out by using silica bound rose Bengal,[31] same products were obtained but the reaction was observed to be slow.

The structure of the photoproducts was assigned on the basis of IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral and elemental analysis studies. The spectral data of photoproducts **2, 3** and **4** were found to be similar with that of **1** except for the furan signals. The furan ring has been site of attack is evident from absence of carbon /hydrogen signals due to furan moiety in the spectral data of all the identified photoproducts. The spectral studies suggested that product **2** posses δ-hydroxy butenolide moiety instead of furan moiety. The additional IR bands at 3390 cm^{-1} (hydroxy group), 1670 (α, β-unsaturated ketone) and extra carbonyl ^{13}C- resonance at δ 175.7 suggested an extra lactone carbonyl compared to that of parent compound. The ^{13}C-NMR signal for C-5' at δ 97.5ppm (attached to proton at a downfield value of δ 6.15 ppm) indicated that carbon is attached to two oxygen atoms. This proton is in split

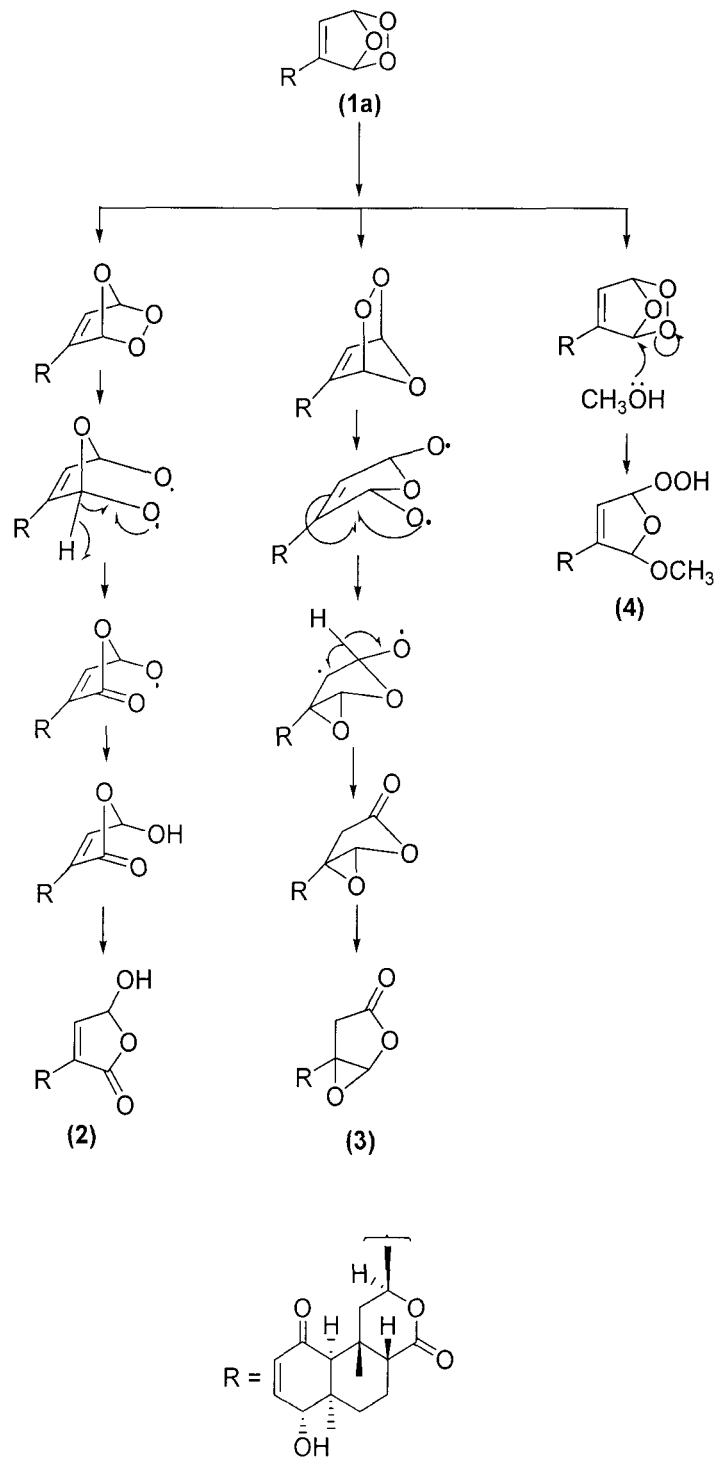
with the proton of α, β-unsaturated carbon at δ 6.94, suggesting that carbon attached to two oxygen atoms must be adjacent to α, β-unsaturated carbon. The change of furan ring to δ-hydroxy butenolide moiety is also evident from the two carbon signals at δ 136.5 and 175.7 instead of the olefinic signals of furan at δ 121.5 and 139.7.

The spectral data for compound **3** was also found to be similar with that of **1** except for the furan signals. On the basis of following spectral data we conclude the presence of epoxy lactone in **3**. The ^{13}C-NMR spectrum indicated an additional lactone carbonyl resonance at δ 171.1. A signal at δ 120.4 was assigned to a dioxygenated carbon of the epoxylactone ring, with additional support from its ^{1}H-NMR signal at δ 5.23. Of the other carbons of the lactone ring, a carbon resonance value at δ 36.2 along with a proton resonance at δ 2.2 was assigned to the methylene carbon and at δ 59.2 assigned to the quaternary carbon. The formation of lactone ring gets additional support from the IR spectrum of **3**, which shows characteristic absorption for two lactone carbonyl at 1750 and 1710 cm^{-1} and for the epoxide ring at 3150, 1210, 950 and 745 cm^{-1}.

The compound **4** was having a comparably similar spectral data to **1,** with a basic difference in furan ring values. It was shown to contain a 2,5-dihydrofuran ring with an allylic hydroperoxy and a methoxy group. The ^{1}H-NMR spectrum recorded a highly deshielded signal at δ 8.1 (brs, exch., 1H) and a three proton singlet at δ 3.32, which were assigned to the allylic –OOH

group (at C-5') and –OMe group (at C-2') respectively. This regiostructure gets support from the ^{1}H-NMR signals as a singlet at a low value of δ 5.84 for C-2' proton and a doublet at a high value of δ 6.21 for C-5' proton. Of the olefinic carbons, a carbon resonance at δ 114.7 was assigned to C-4' and at δ 141.4 was assigned to C-3'. The signals for the protons at C-4' and -OCH_3 were appropriately observed at δ 5.72 and 3.32, respectively. Additional structural information for the compound **4** was inferred from its following chemical properties: 1) with $Pb(OAc)_4$, gas was evolved, which is characteristic of compounds containing -OOH group; 2) with potassium iodide-acetic acid solution it liberated iodine, indicating presence of O-O bond.[32]

The formation of photoproducts **2**, **3** and **4** can be envisaged to occur from unstable cyclic peroxide **1a**, which initially results by a [4π+2π] cycloaddition of $^1O_2$ to furan ring (Scheme 5.1). This unstable cyclic peroxide **(1a)** undergoes homolytic cleavage of O-O bond to give a diradical which affords products **2** and **3** by following two competing processes:[33,34] in one way the diradical intermediate formed undergos epoxycyclization followed by 1,2-hydrogn shift gives compound **3**. In an alternative competitive path a 1,4-hydrogen migration in the diradical intermediate gives product **2** (Scheme 5.2). In the presence of polar methanol solvent, the solvolysis induced transformation of intermediate **1a** leads to the formation of product **4**.



Scheme 5.2

[B] Photooxidation of 2β-angeloyloxy-10β-H-furanoeremophilane

Furanoeremophilanes, a novel class of sesquiterpenes is the constituents of several medicinal plants[36-38] and is well known for their medicinal values[39-41] e.g. antioxidant and antiradical property, toxicity[42,43] and antifeedant[44] activity. Herein we have investigated photooxidation of 2β- angeloyloxy-10β-H-furanoeremophilane[45] **(5)** in its reaction with singlet oxygen ($^1O_2$) using different reaction media.

Experimental

Instrumentation and chemicals

Same as in section [A]

Irradiation procedure

Irradiation of 2β-angeloyloxy-10β-H-furanoeremophilane (5) in benzene

2β-Angeloyloxy-10β-H-furanoeremophilane was isolated as described in the literature.[45] Compound **5** (100 mg , 0.316 mM) was dissolved in benzene (250 ml) and the solution was irradiated, under continuous bubbling of air, with a light from a 400W medium pressure mercury lamp housed in a water cooled immersion well quartz photo-reactor. The Progress of reaction was monitored by thin layer chromatography (TLC), which indicated gradual disappearance of starting material. When the rate of product formation became negligible, solvent was removed and the residue was purified by TLC on silica gel, eluting

with 50% ether-hexane, where it yielded hydroxybutenolide **(8)** and epoxylactone **(10)** as the products (Scheme 5.3).

2β-Angeloyloxy-8-hydroxy-10β-H-eremophilanolide (8): Yield: 38.48 mg; mp 210° C; HRMS calcd. for (M^+) $C_{20}H_{28}O_5$ 348.198 found 348.199; IR ν_{max}^{cm-1}: 3620 (-OH), 1765 (Lactone), 1715, 1650 (C=C-COOR); ^{1}H-NMR ($CDCl_3$) δ 1.06 (d, J=6.8 Hz , 3H, H-15), 1.16 (s, 3H, H-14), 1.41 (s, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.55 (dd, J=17 & 9 Hz, 1H, H-9), 1.59 (m, 1H, H-4), 1.71 (d, J=1.5 Hz, 3H, H-4'), 1.75 (s, 1H, H-6), 1.76 (m, 2H, 1-H & 3-H), 1.80 (d, J=17& 9 Hz, 1H, 9-H), 1.93 (s, 6H, H-5' & H-13), 2.00 (s, 1H, H-6), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'); ^{13}C-NMR ($CDCl_3$) δ 11.6 (C-13), 12.1 (C -4'), 16.4 (C-15), 17.5 (C -5'), 20.5 (C-14), 25.4 (C-10), 26.5 (C-6), 31.1 (C-4), 35.1 (C-1), 37.3 (C-3), 40.6 (C-9), 50.8 (C-5), 71.8 (C-2), 110.9 (C-8), 125 (C-11), 128.3 (C-2'), 138.6 (C-3'), 156.3 (C-7), 167.2 (C-1') 176.0 (C-12); EI-MS m/z (rel. int.%): 349 (M+1, 25), 245 (M+1-RCOOH, 42), 218 (100).

2β-Angeloyloxy-7,8-epoxy-10β-H-eremophilanolide (10): Yield: 19.75 mg; mp 206 °C; HRMS calcd. for (M^+) $C_{20}H_{28}O_5$ 348.198 found 348.197; IR ν_{max}^{cm-1} : 1765 (Lactone), 1715, 1650 (C=C-COOR) 1420, 1380, 1330, 1293, 1171, 964, 816, 733, 562; ^{1}H-NMR ($CDCl_3$) δ 1.06 (d, J=6.8 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.24 (d, J=7 Hz, 3H, H-13), 1.33 (dd, J= 15 & 7 Hz, 1H, H-6), 1.41 (s, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.58 (dd, J= 15 & 7 Hz, 1H, H-6), 1.59 (m, 1H, H-4), 1.64 (dd, J=15 & 7 Hz, 1H, H-9), 1.71 (d, J=1.5 Hz, 3H, H-4'), 1.76 (m, 2H, H-1 & H-3), 1.89 (dd, J=15 & 7, 1H, H-9) 1.93 (s, 3H, H-5'), 2.78

(q, J=7 Hz, 1H, H-11), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'); ^{13}C-NMR ($CDCl_3$) δ 10.3 (C-13), 12.1 (C-4'), 16.3 (C-15), 17.5 (C -5'), 20.1 (C-5), 27.4(C-10), 30.5 (C-9), 30.7(C-4), 34.4 (C-1), 36.7 (C-6), 37.2 (C-3), 71.8 (C-2), 93.3 (C-8), 128.3 (C-2'), 138.6 (C-3'), 167.2 (C-1'), 177.4 (C-12); EI-MS m/z (rel. int.%): 347 (M^+, 1), 329 (M^+-H_2O, 2), 247 (M^+-C_4H_7COOH, 12), 83 ($C_4H_7CO^+$, 100), 55 (83-CO, 44).

Photosensitized oxygenation of 2β-angeloyloxy-10β-H-furanoeremophilane (5) in methanol

Compound **5** (100 mg, 0.316 mM), was dissolved in 250 ml MeOH containing 100 mg of rose bengal. The solution was irradiated with a 400W medium pressure mercury lamp in a water-cooled immersion well type quartz photo reactor with continuous supply of O_2. The progress of the reaction was monitored by TLC (silica gel, ether-hexane). When the rate of product formation became negligible solvent was evaporated in a rotary evaporator, and the residue taken up in ether, the ether was washed with water, treated with activated charcoal, dried and evaporated to yield **11** as colorless oil (Scheme 5.4).

2β-Angeloyloxy-10β-H-8-methoxy-12-hydroperoxy dihydrofuranoeremophilane (11): Yield: 37.20 mg; mp 198° C; HRMS calcd. for (M^+) $C_{21}H_{32}O_6$ 380.219 found 380.220; IR ν_{max}^{cm-1}: 3514, 2130, 1830, 1715, 1650, 1250; ^{1}H-NMR ($CDCl_3$) δ 1.06 (d, J=6.8 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.41 (m, 1H, H-10), 1.42 (dd, J= 15 & 7 Hz, 1H, H-9), 1.57 (m, 2H,

H-1), 1.51 (m, 2H, H-3), 1.59 (m, 1H, H-4), 1.67 (dd, J= 15 & 7 Hz, 3H, H-9), 1.71 (d, J=1.5 Hz, 3H, H-4'), 1.75 (dd, J=15 & 7 Hz, 1H, H-6), 1.76 (m, 2H, H-3), 1.81 (d, J=1.5 Hz, 3H, H-13), 1.93 (s, 3H, H-5'), 3.24 (s, -0CH_3), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'), 8.16 (s, 1H, H-12); ^{13}C-NMR ($CDCl_3$) δ 7.6 (C-13), 12.1 (C-4'), 16.3 (C-15), 17.5 (C-5'), 20.5 (C-14), 21.2 (C-6), 25.8 (C-10), 35.4 (C-1), 37.3 (C-3), 39.5 (C-9), 51.4 (C-5), 51.6 (-OCH_3), 71.8 (C-2), 110.3 (C-12), 112.0 (C-8), 128.3 (C-2'), 130.8 (C-11), 138.6 (C-3'), 141.7 (C-7), 167.2 (C-1'); EI-MS m/z (rel. int.%): 381 (M^+, 7), 363 (M-H_2O, 13), 209 (15), 180.15 (3), 83 (100).

Rearrangement of 11 under acidic condition

Compound **11** (0.2 mM) was taken in MeOH to which 5% HCl was added until the solution became cloudy. The mixture was refluxed for 2 hr, cooled, diluted with water, and extracted with ether to yield a compound identified as **8**. All the spectral values in IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra were found to correspond to that of **8** (Scheme 5.5).

Pyrolysis of Photoproduct 11

A sample of **11** (0.2 mM) was taken in benzene and injected into the gas chromatograph (column, 200°, injection block 250°). A single product as **12** was formed. The product was collected from the gas chromatograph (Scheme 5.5).

2β-Angeloyloxy -10β-H-8-methoxy-eremophilanolide (12): Yield: 31.15 mg; UV λ_{max} 216 nm; mp 200° C; HRMS calcd. for (M^+) $C_{21}H_{30}O_5$ 362.208 found 362.209; IR ν_{max}^{cm-1}: 1779, 1760, 1698, 1650, 1533; ^{1}H-NMR ($CDCl_3$) δ 1.06 (d, J=6.8 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.41 (m, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.53 (dd, J=15 & 7 Hz, 1H, H-9), 1.59 (m, 1H, H-4), 1.71 (d, J= 15 Hz, 3H, H-4'), 1.75 (dd, J=15 & 7 Hz, 1H, H-6), 1.76 (m, 2H, H-1 & H-3), 1.78 (dd, J=15 & 7 Hz, 1H, H-9), 2.00 (dd, J=15 & 7, 1H, H-6), 3.24 (s, -OCH_3), 3.91 (m, 1H, H-2), 6.03 (m, 1H, H-3'); ^{13}C-NMR ($CDCl_3$) δ 7.6 (C-13), 12.1 (4'-C), 16.4 (C-15), 17.5 (C-5'), 20.5 (C-14), 21.2 (C-6), 25.8 (C-10), 31.1 (C-4), 35.4 (C-1), 39.5 (C-9), 51.4 (C-5), 51.6 (-OCH_3), 71.8 (C-2), 110.3 (C-12), 112.0 (C-8), 128.3 (C-2'), 130.8 (C-11), 138.6 (C-3'), 141.7 (C-7), 167.2 (C-1'); EI-MS m/z (rel. int.%): 363 (M+, 34), 332 (M^+-OCH_3, 13), 263 (M^+-C_4H_7COOH, 11), 83 ($C_4H_7CO^+$, 93), 55 ($C_4H_7CO^+$-CO, 40).

Reduction of 11 with triphenylphosphine

A solution of **11** (0.2 mM) in ether was added drop wise to a refluxing solution of triphenylphosphine in 30 ml ether during 1.5 h. The solution was refluxed 1 h, chilled to -5 ^{0}C and filtered to remove the triphenylphosphine oxide. The ether was removed with a rotary evaporator, and the residue was chromatographed on silica gel column to give **16** (Scheme 5.5).

2β-Angeloyloxy -10β-H-eremophilanolide (16): Yield: 31.85 mg; UV λ_{max} 217 nm; mp 195° C; HRMS calcd. for (M^+) $C_{20}H_{28}O_4$ 332.198 found 332.199; IR ν_{max}^{cm-1}: 1808, 1800, 1765, 1715, 1650, 1000; ^{1}H-NMR ($CDCl_3$): δ 1.06 (dd,

J=15 & 7 Hz, 3H, H-15), 1.16 (s, 3H, H-14), 1.40 (dd, J=15 &7 Hz, 1H, H-9), 1.41 (m, 1H, H-10), 1.51 (m, 2H, H-1 & H-3), 1.59 (m, 1H, H-4), 1.65 (dd, J=15 & 7 Hz, 1H, H-9), 1.75 (d, J=17 Hz, 1H, H-6), 1.76 (m, 2H, 1H & H-3), 1.93 (s, 3H, H-5'), 1.93 (s, 3H, H-13), 3.91 (m, 1H, H-2), 4.91 (dd br, J=6.5 & 10 Hz, 1H, H-8), 6.03 (m, 1H, H-3'); ^{13}C-NMR ($CDCl_3$): δ 11.3 (C-13), 12.1 (C-4'), 16.4 (C-15), 17.5 (C-5'), 20.5 (C-14), 31.1 (C-4), 31.6 (C-10), 32.7 (C-6), 34.0 (C-9), 34.8 (C-1), 37.3 (C-3), 50.5 (C-5), 71.8 (C-2), 81.2 (C-8), 125.9 (C-11), 128.3 (C-2'), 138.6 (C-3'), 164.6 (C-7), 167.2 (C-1'), 176.0 (C-12); EI-MS m/z (rel. int.%): 333 (M^+, 37), 277 (M^+-C_4H_7, 24), 233 (M^+-C_4H_7COOH, 31), 83 ($C_4H_7CO^+$, 91), 55 ($C_4H_7CO^+$-CO, 13).

Irradiation of 5 in Silica gel bound Rose bengal

Compound **5** (100 mg, 0.316 mM), [P_{si}]–rose bengal[31] (200 mg, 6.5 mg/g) and 100 ml of methanol were placed in the photochemical reactor and irradiated at 10° C in the presence of bubbling oxygen. The progress of reaction was monitored by TLC. After 10 h of irradiation, the reaction mixture was removed, washed with methanol and chromatographed on silica gel to give two products, identified to be same as **8** and **10** by comparison of their spectral data. It was found that upon standing the reaction mixture and so also on addition of dil HCl in the reaction mixture, the product **10** (epoxy lactone) converted into hydroxy butenolide **8** (Scheme 5.4).

Result and Discussion

Irradiation of furanoeremophilane **(5)** in benzene under continuous air bubbling with quartz filtered light from a medium pressure mercury lamp, and purification of the crude product by silica gel column chromatography afforded compound **8** and **10**, identified as 2β-angeloyloxy-8-hydroxy–10β-H-eremophilanolide and 2β-angeloyloxy–7,8-epoxy-10β-H-eremophilanolide, respectively. Both the 2β-angeloyloxy-10β-H-furanoeremophilane **(5)** and hydroxybutenolide **(8)** have been isolated from the same plant species and it has been indicated that **5** is probable natural artefact of **8**.[45]

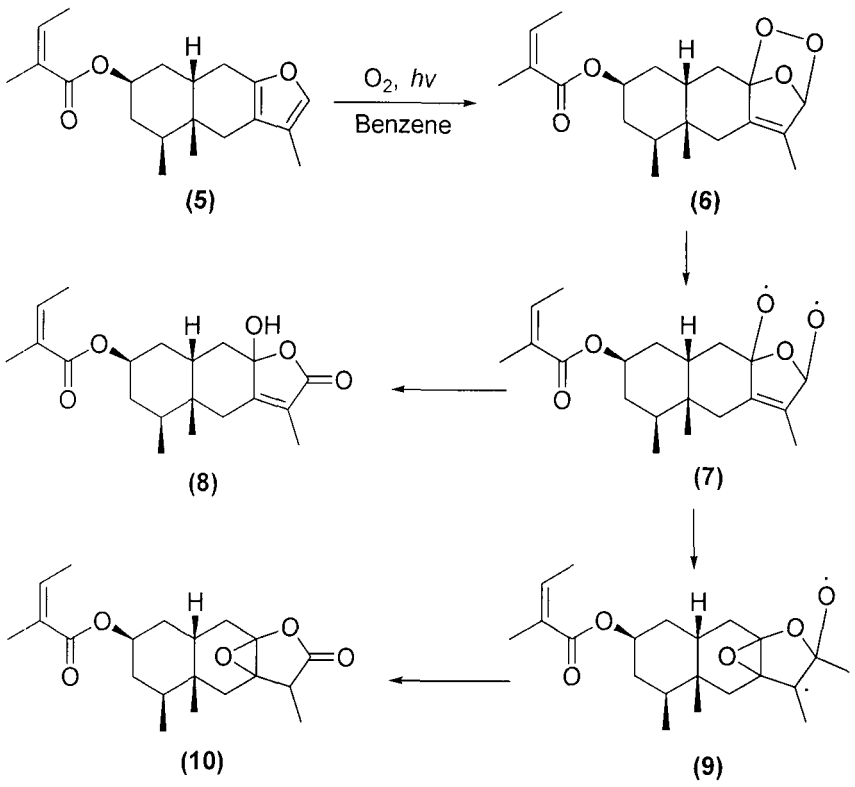
The mechanism of formation of γ-hydroxybutenolide **(8)** and epoxylactone **(10)** is depicted in Scheme 5.3. [4π+2π] Cycloaddition of $^1O_2$ to furan moiety of **5** gives an unstable ozonide peroxide intermediate **(6)**, which by homolytic cleavage of O-O bond produces diradical intermediate **7**. Intermediate **7** on epoxycyclization followed by 1,2 – hydrogen shift gives compound **10**. In an alternative competitive path a 1,4-hydrogen migration in the intermediate **7** gives product **8** (Scheme 5.3). Cyclic peroxides are generally unstable, however in some cases stable peroxides have been isolated.[46] The participation of $^1O_2$ in this reaction was confirmed by studying the effect of DABCO (singlet oxygen scavenger) on the yields of photooxidation products. The drastic lowering of the yield of products in presence of DABCO confirms that $^1O_2$ is active oxidizing species in this photoreaction. Also no reaction was observed on conducting experiments under nitrogen atmosphere.

The ^{1}H-NMR and ^{13}C-NMR spectrum of compound **8** were similar to those of **5** except for the furan signals. The extra carbonyl resonance at δ 177.4 ppm indicated an additional lactone carbonyl compared to that of parent compound. This was confirmed by the presence of IR bands at 1765, 1715 and 1650 cm^{-1}. The absence of C/H NMR signals due to furan moiety indicated that the furan ring had been the site of attack. ^{13}C-NMR signals at δ 110. ppm (carbon having no proton), indicated that the carbon must be attached to two oxygen atoms.

Further, δ 2.48 and 2.23, the H-9 signal in compound **5** changes to δ 1.80 and 1.55 ppm, suggesting that double bond between C_7-C_8 in **5** is shifted to C_7-C_{11} in **8**, and the carbon connected to two oxygen atoms must be adjacent to β carbon of the α, β-unsaturated ketone system. The presence of other carbon signals at 177.4, 156.3, 125.9 and 11.6 ppm along with an IR band at 3620 cm^{-1} indicated that the furan ring has been modified to a γ-hydroxybutenolide moiety. The compound was thus assigned structure as **8** with a molecular formula $C_{20}H_{28}O_5$ (M^+, 349).

The spectral data of photoproduct **10** was almost identical to that of starting compound **5**, except for the values corresponding to an epoxide at C_7-C_8 and an epoxylactone in place of furan ring. This is evidenced by the following changes in the methylene carbon signals: δ 2.60, 2.35 ppm (C-6) and δ 2.48, 2.23 ppm (C-9), changed to δ 1.58, 133 ppm (C-6) and δ 1.89, 1.64 ppm (C-9) suggesting that the change has occurred at C_7-C_8. Further ^{13}C-NMR value at δ 59.3 ppm

(C-7) and 93.3 ppm (C-8), suggested that initially sp^2 hybridized carbon changed to quaternary carbon. The compound **10** showed a proton singlet at δ 2.78 ppm, attached to C-11 (δ 47.8 ppm), which was not present in the in starting compound. This suggested that both the double bonds of furan ring were utilized in epoxide and lactone formation. ^{13}C-NMR exhibited signal due to lactone carbonyl at δ 177.4 ppm which is supported by the IR bands at 1765 cm^{-1} (lactone) and 1715 cm-1 (α, β-unsaturated ester).

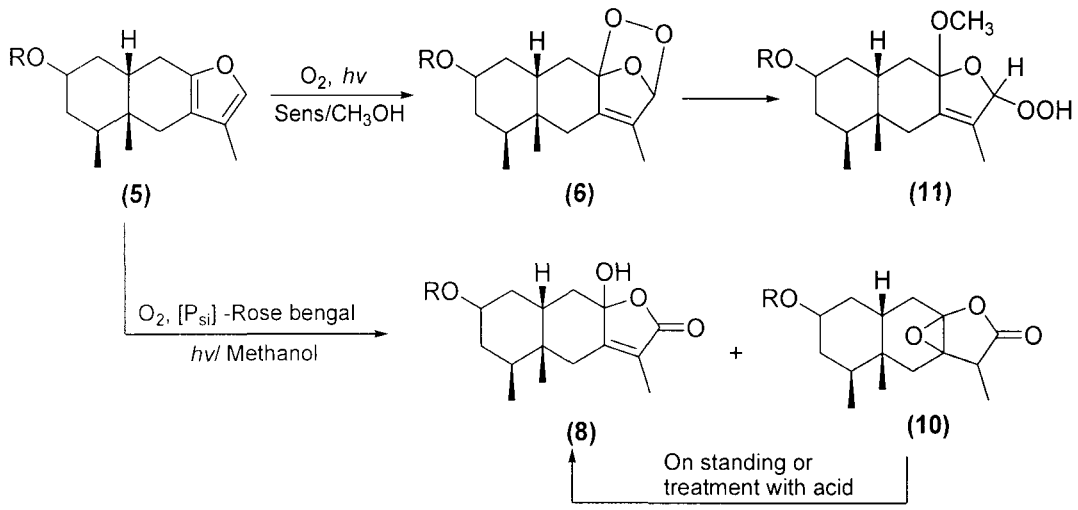


Scheme 5.3

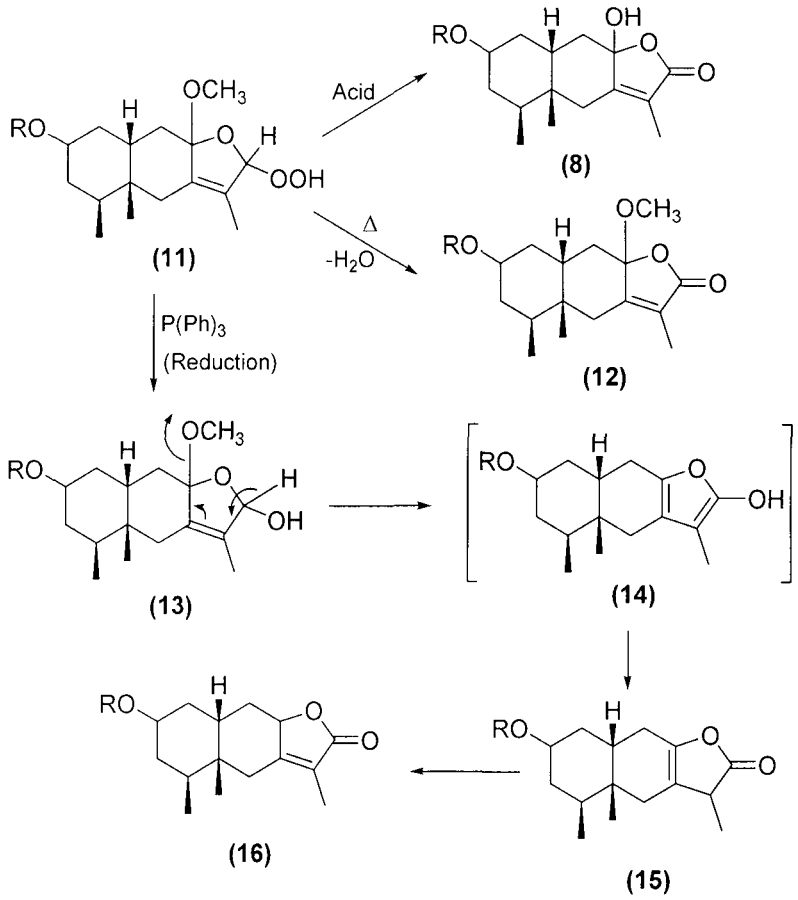
Photooxygenation of **5** in methanol gave a compound identified as a crystalline hydroperoxide whose properties require that it should have structure **11**. The compound has absorption bands at 3514 cm^{-1} (-OOH) and 1250 cm^{-1} (C-O) but none in –C=O region indicating it to be a hydroperoxide. Its ^{1}H-NMR spectrum has significant signals at δ 8.16 (1H, OOH, exch.) and δ 3.24 (3H, OCH_3) consistent with structure **11**. A quantitative Zeisel determination indicated the presence of one OMe group and the result of quantitative peroxide and active hydrogen determinations were consistent with the presence of one O-O and one OH group. When **5** was irradiated in presence of [P_{si}]–rose bengal[31] in methanol under bubbling oxygen a mixture of products **8** and **10** was obtained. It was found that upon standing the reaction mixture and so also on addition of dil HCl in the reaction mixture, the product **10** (epoxy lactone) converted into hydroxy butenolide **8** (Scheme 5.4).

Treatment of methoxy hydroperoxide **11** with methanolic HCl gave a product identified as **8**. Whereas pyrolysis of **11** produced **12** (Scheme 5.5). The structure of **12** was readily established by its spectral and chemical properties. In the IR, the compound absorbs at 1779 cm^{-1}, characteristics of γ-oxygenated α,β-unsaturated-γ-lactone functionality. In the UV as well, the absorption characteristics of this chromophore occurred at λ_{max} 216 nm. The NMR spectrum clearly showed the presence of a –OMe group δ 3.24 (3H) and an allylic Me δ 1.71 (3H). The presence of the OMe group was confirmed by a quantitative zeisel determination. Reduction of **11** with triphenylphosphine in

ether gave a product which was identified as **16**. Its formation could be realized via unstable hemiacetal **14** (Scheme 5.5). Its IR absorption at 1779 cm^{-1} and UV λ_{max} 217 nm are characteristic of an α, β-unsaturated γ-lactone. The NMR spectrum showed the presence of an allylic Me group, δ 1.93 (3H, t); the single proton in the lactone ring appears as the quartet centered at δ 4.91 (1H, J=6 and 11 Hz). The compound was identified as 2β-angeloyloxy -10β-H-eremophilanolide **(16)**.


RO
H
O
O2, hv
Sens/CH3OH
(5)
(6)
(11)
OCH3
OOH
O2, [Psi] -Rose bengal
hv/ Methanol
OH
(8)
(10)
On standing or
treatment with acid

Scheme 5.4


RO
H
OCH3
O
H
OOH
(11)
Acid
RO
H
OH
O
O
(8)
Δ
-H2O
RO
H
OCH3
O
O
(12)
P(Ph)3
(Reduction)
RO
H
OCH3
O
H
OH
(13)
RO
H
O
OH
(14)
RO
H
O
O
(15)
RO
H
O
O
(16)

Scheme 5.5

References

1. N. R. Farnsworth, O. Akerele, A. S. Bingel, D. D. Soejarto, Z. Guo, *Bull. WHO.*, 1985, **63**, 965.
2. G. M. Cragg, D. J. Newman, K. M. Snader, *J. Nat. Prod.*, 1997, **60**, 52.
3. A. Kuruvilla, *Indian J. Exp. Biol.*, 2002, **40**, 7.
4. P. M. Dewick Medicinal Natural Products, A Biosynthetic approach, 2nd Edition, John Wiley and Sons, New Jersey, 2001.
5. G. M. J. Beijersbergen Van Henegouwen, B. Testa U. Meyer Ed., Advances in Drug Research, Academic, New York and London, 1997, 29, p 79.
6. M. A. Miranda, In Vitro Methods in Pharmaceutical Research, J. V. Castell Ed., Academic Press, London, 1997, p 289.
7. Symposium-in print Highlights- The importance of drug photostability, photochemistry and phototoxicity. Photochemistry and Photobiology Focus: November 1998, Vol. 68, 5.
8. A. Albini, E. Fasani, Drugs: Photochemistry and Photostability, Royal Society of Chemistry, Cambridge, 1998.
9. E. D. Morgan, A. P. Jarvis, G. R. Jones, *ARKIVOC*, 2000, **1**, 312.
10. S. Walia, S. K. Kulshrestha, S. K. Mukherjee, *Tetrahedron*, 1986, **42**, 4817.

11. Wu, Shuping, J. Zhiqin, Li Heting, Y. Li, Z. Daixun, *Molecules*, 2001, **6**, 52.

12. K. -S. Ngo, G. D. Brown, *Tetrahedron*, 1999, **55**, 15109.

13. S. MacKinnon, T. Durst, J. T. Arnason, C. Angerhofer, J. Pezzuto, P. E. Sanchez-Vindas, L. J. Poveda, M. Gbeassor, *J. Nat. Prod.*, 1997, **60**, 336.

14. D. H. Bary, J. D. Connolly, M. J. O. Neill, J. D. Phillipson, D. C. Warhurst, *Phytotherapy Res.*, 1990, **4**, 29.

15. J. P. Knox, A. D. Dodge, *Phytochemistry* 1985, **24**, 889.

16. Y. R. Chaddha, The Wealth of India, Publication and information directorate, CSIR, New Delhi, 1976, 10, p 251.

17. B. Bishayi, S. Roychowdherry, S. Ghosh, M. Sengupta, *J. Toxicol. Sci.*, 2002, **27**, 139.

18. P. S. Prince, V. P. Menon, *J. Ethnopharmacol.*, 1999, **65**, 277.

19. G. C. Jagetia, K. Nayak, M. S. Vidyasagar, *Cancer Lett.*, 1998, **127**, 71.

20. N. N. Rege, S. A. Dahanukar, *J. Postgrad. Med.*, 1993, **31**, 22.

21. R. K. Bhatt, J. B. Hanuman, B. K. Sabata, *Phytochemistry*, 1988, **27**, 1212.

22. V. Wazir, R. Maurya, R. S. Kapil, *Phytochemistry*, 1995, **38**, 447.

23. J. B. Hanuman, R. K. Bhatt, B. K. Sabata, *Phytochemistry*, 1986, **25**, 1677.

24. R. Maurya, V. Wazir, A. Tyagi, R. S. Kapil, *Phytochemistry*, 1995, **38**, 659.

25. D. R. Kearns, Singlet Oxygen, H. H. Wasserman, R. W. Murray Ed., Academic, New York, 1979, p 120.

26. K. Gollnick, H. J. Kuhn, Singlet Oxygen, H. H. Wasserman, R. W. Murray Ed., Academic Press, New York, 1979, p 290.

27. E. M. Tuite, J. M. Kelly, *J. Photochem. Photobiol. B: Biol.*, 1993, *21*, 103.

28. S. Roul, J. Cadet, *J. Am. Chem. Soc.*, 1996, **118**, 1892.

29. C. S. Foote, *Photochem. Photobiol.*, 1991, **54**, 659.

30. J. Cadet, C. Decarroz, S. Y. Wang, W. R. Midden, *Isr. J. Chem.*, 1983, **23**, 420.

31. S. Tamagaki, C. E. Liesner, D. C. Neckers, *J. Org. Chem.*, 1980, **45**, 1573.

32. C. S. Foote, M. T. Wuesthoff, S. Wexler, I. G. Binstain, R. Denny, *Tetrahedron*, 1967, **23**, 2583.

33. M. Balci, *Chem. Rev.*, 1981, **81**, 91.

34. K. Gollnick, A. Griesbeck, *Tetrahedron*, 1985, **41**, 2057.

35. J. D. Coyle, Photochemistry in Organic Synthesis, Burlington house, London, 1986, p 191.

36. R. J. Nachman, *Phytochemistry*, 1983, **22**, 780.

37. M. Mao, Z. Yang, Z. Jia, *Planta Medica*, 2003, 745.

38. P. Torras, J. Ayala. C. Grande, J. Anaya M. Grande, *Phytochemistry*, 1999, **52**, 1507.

39. W. D. Inman, S. R. King, J. L. Evans, J. Lu, *U. S. Patent 5,* 1998, 747.

40. N. P. Krasouskaya, N. I. Kullesh, V. A. Denisenko, *Chem. Nat. Compd. (Engl Transl.)*, 1989, **25**, 545.

41. S. P. Visnoi, A. Shoeb, R. S. Kapil, S. P. Popli, *Phytochemistry*, 1983, **22**, 597.

42. F. Bohlman, K. H. Knoll, C. Zedro, P. K. Mahanta, M. Grenz, A. Sunita, D. Ehlens, N.-L. Van, W.-R. Abraham, A.-N. Natu, *Phytochemistry*, 1977, **16**. 965.

43. P. W. Jennings, J. C. Hurly, S. K. Reeder, A. Holian, P. Lee, C. N. Caughlan, R. D. Larsen, *J. Org. Chem.*, 1976, **41**, 4078.

44. A. Coloma, M. Reina, R. Cabrera, P. Castanera, C. Gutierrez, *J. Chem. Ecol.*, 1995, 1225.

45. F. Bohlmann, J. Ziesche, *Phytochemistry* 1980, **19**, 2681.

46. K. Gollinck, A. Griesbeck, *Tetrahedron*, 1985, **41**, 2057.